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THE EFFECTS OF
KAPPA OPIOID AND DOPAMINE AGONISTS ON
UNCONDITIONED BEHAVIORS AND FOS IMMUNOREACTIVITY
IN PREWEANLING AND ADULT RATS

A Thesis
Presented to the
Faculty of
California State University,
San Bernardino

In Partial Fulfillment
of the Requirements for the Degree
Master of Arts
in
Psychology

by
Marcus Alan Duke

June 1996


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KAPPA OPIOID AND DOPAMINE AGONISTS ON
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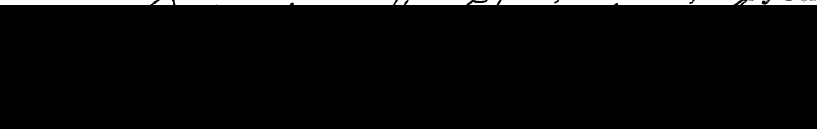
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June 1996

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6/19/96
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ABSTRACT

The kappa opioid agonist U-50,488 is known to decrease the locomotor activity of adult rats. In contrast, U-50,488 dramatically increases the locomotor activity of preweanling rats. Although the mechanisms responsible for U-50,488's paradoxical effects are uncertain, it is possible that U-50,488 was increasing locomotor activity by activating a dopaminergic process. To assess this kappa/DA interaction 17- and 80-day-old rats were treated with the DA antagonist flupenthixol (0.025--0.8 mg/kg, i.p.) prior to an injection of U-50,488 (5.0 mg/kg, s.c.). Flupenthixol did block U-50,488-induced activity, but it is possible that this was due to a general depression in motoric functioning. Therefore, the effects of U-50,488, NPA (a direct DA agonist), and amphetamine (an indirect DA agonist) were assessed in 17- and 80-day-old rats. Consistent with previous studies, U-50,488 increased the locomotor activity of preweanling rats, while having no effect on the activity of adult rats. NPA attenuated U-50,488-induced locomotor activity, in a dose-dependent manner, while amphetamine had few effects on U-50,488's activity enhancing effects. In these experiments Fos was assessed in order to determine the specific brain regions where U-50,488 was working. Fos immunohistochemistry indicated that U-50,488 increased

Fos expression in several brain regions of the preweanling rat, including: olfactory tubercles, habenula, striatum, and preoptic area. Importantly, NPA reduced U-50,488-induced Fos induction in the striatum. In an additional set of experiments, cross-sensitization was assessed to further examine the kappa/DA interaction in the preweanling rat. At 11 days of age, rats were given an injection of amphetamine or NPA for 5 consecutive days. Following a two day interval (at 17 days of age), rats received a challenge injection of U-50,488 or the same DA agonist again. There was no cross-sensitization between U-50,488 and either of the DA agonists, suggesting that U-50,488's locomotor activating effects are not directly mediated through a dopaminergic mechanism. Therefore, when taken together these results suggest that U-50,488's ability to paradoxically increase the locomotor activity of preweanling rats is not due to actions on DA neurons. Rather, the kappa opioid and DA systems appear to have an antagonistic relationship, as kappa opioid and DA agonists reciprocally inhibit one another.

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I extend a special THANK YOU to Sandy (HIM)! I truly feel combat ready--at this point hell would be a picnic. You knew every button to push and chain to yank. Contrary to what my actions often indicated, I do value and respect your advise and guidance. I feel very fortunate to consider you as not only my mentor, but also my friend. If I may leave you with one thought concerning our years together: performance is not always a true measurement of learning.

Cynthia, I thank you for your help and moral support. I only regret not having had the opportunity to work in the lab together more than we did. This is for you Cynthia: The 1996 NCAA National Basketball Champion University of Kentucky Wildcats, *GO BIG BLUE!*

I also want to thank the CREW: Torren, Carlos, Teri, and Rob. I appreciate the many hours and the comic relief. Lastly, to my friend Scott (plus Arnie, Banda, and SKIZILLA), thanks for letting me hang out at the asylum. Life on the hill made all this possible.

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Introduction

Ontogenetic Research Strategy

The ontogenetic research strategy has become an increasingly popular approach for studying the neurobiological mechanisms underlying behavior. Besides the obvious advantage of enhancing our knowledge of the developing organism, the ontogenetic strategy provides an additional tool for determining those mechanisms or systems mediating behavior. More specifically, as the animal matures new behaviors emerge as the underlying neurobiological mechanisms become functional. Hence, the ontogenetic process can itself be used as a tool to pinpoint those receptor or neurotransmitter systems mediating a particular behavior (see Spear, 1979; Zolman & McDougall, 1983, for additional reviews).

Recently, a number of different laboratories have used the ontogenetic research strategy to study the mediation of behavior by the dopamine and kappa opioid receptor systems. In each case, these receptor systems have been found to exhibit both quantitative and qualitative changes across development. Quantitative changes are the most common, as drug responsiveness often shows a monotonic increase according to age. For example, dopamine agonists (e.g., apomorphine and quinpirole) increase the locomotor activity of rats as young as 4 days of age (Camp & Rudy, 1987; McDougall,

Arnold, & Nonneman, 1990; Moody & Spear, 1992; Shalaby & Spear, 1980). Apomorphine and quinpirole induce identical behaviors in older rat pups and adults, but the potency of these dopamine-acting drugs increases linearly with age (Moody & Spear, 1992; Shalaby & Spear, 1980).

Of more interest, are those psychopharmacological effects which exhibit qualitative changes across development. For instance, the dopamine mediated behaviors of preweanling rats are sometimes characterized by the emergence of new behaviors (e.g., grooming), and the occurrence of age-specific behaviors (e.g., wall climbing), which are only found during a restricted period of development (Moody & Spear, 1992; Shalaby & Spear, 1980). The kappa opioid system also shows qualitative ontogenetic differences, as high doses of U-50,488 (a kappa agonist) *decrease* the locomotor activity of adult rats and mice (Hayes, Skingle, & Tyers, 1985; Jackson & Cooper, 1985; Leighton, Johnson, Meecham, Hill, & Hughes, 1987; Ukai & Kameyama, 1985; Von Voightlander, Lahti, & Ludens, 1983). In contrast, high doses of U-50,488 *increase* the locomotor activity and wall-climbing of preweanling rats (Bolanos, Garmsen, Clair, McDougall, & Crawford, 1995; Jackson & Kitchen, 1989).

Summary and Proposal. When considered together it

appears that both the kappa opioid and DA systems induce behavioral patterns which vary both quantitatively and qualitatively across development. Curiously, kappa opioid agonists and DA agonists have similar behavioral effects in preweanling rats. This suggests that in these younger animals, kappa neurons may in some way modulate those DA systems involved with unlearned activity.

The purpose of this project was to assess the possible interaction between the DA and kappa opioid systems in the preweanling and adult rat. To this end, 17- and 80-day-old rats were treated with a variety of direct and indirect DA agonists and antagonists, as well as a kappa opioid agonist. Fos immunohistochemistry, was used to identify those pathways activated by the various drugs. This study should determine: first, the behaviors elicited by kappa opioid and DA agonists in both adult and preweanling rats; second, whether the kappa and DA systems interact to mediate behavior; and third, the neuroanatomical regions activated by kappa and DA agonist treatment.

Dopamine Systems

Dopamine Receptors. Dopamine receptors have been classified into five structurally distinct receptor subtypes: D₁, D₂, D₃, D₄, and D₅ (Clark & White, 1987; Sokoloff, Giros, Martes, Bouthenet, & Schwartz, 1990;

Sunahara et al., 1991; Von Tol et al., 1991). D₁ and D₂ receptors have been differentiated according to behavioral effects, sensitivity to pharmacological agents, anatomical location, and effects on second messenger systems (for a comprehensive review see Clark & White, 1987). For example, the D₁ receptor site increases adenylyl cyclase activity via a G_s protein; whereas, the D₂ receptor site exerts an opposing influence on adenylyl cyclase activity via a G_i protein (Cooper, Bloom & Roth, 1991; Cunningham & Kelley, 1993).

Behavioral effects of dopamine agonists and antagonists in adult rats. DA agonists are typically classified as being either direct or indirect agonists. Direct DA agonists [e.g., R-propylnorapomorphine (NPA) and apomorphine] bind to the post synaptic receptor and mimic the endogenous neurotransmitter. Indirect DA agonists (e.g., amphetamine and cocaine) increase the amount of synaptic DA in a number of different ways. For example, cocaine blocks the reuptake of DA from the synapse; whereas, amphetamine increases DA release into the synapse (Cooper et al., 1991).

Behavioral responses to DA agonists include, but are not limited to, locomotion, sniffing, rearing, and chewing (Arnt, 1987; Clark & White, 1987). Utilization of selective DA receptor agonists has revealed receptor-specific behavioral profiles. D₁ agonists primarily

enhance grooming and non-stereotyped locomotor activity; whereas, D₂ receptor stimulation enhances locomotor activity, rearing, head-down sniffing, and a variety of oral movements (Arnt, Hyttel, & Perregaard, 1987; Dall'Olio, Gandolfi, Vaccheri, Roncada, & Montanaro, 1988; Molloy & Waddington, 1985). The occurrence of these behaviors is largely determined by dose, as a low dose of NPA induces locomotor activity and rearing, while a moderate dose preferentially induces head-down sniffing. Oral behaviors, like chewing, licking, and biting, only occur after higher doses of NPA or apomorphine (Bordi, Carr, & Meller, 1989; McDougall, Crawford, & Nonneman, 1993; Mestlin & McDougall, 1993). Interestingly, although these behaviors are thought to be primarily D₂-mediated, they are only observed when both D₁ and D₂ receptors are activated (Barone, Davis, Braun & Chase, 1986; Longini, Spina & Di Chiara, 1987; Molloy & Waddington, 1985; Robertson & Robertson, 1986). Thus, substantial activation of both D₁ and D₂ receptors is required to produce full expression of highly stereotyped behaviors like licking and chewing.

Not surprisingly, selective or mixed DA antagonists can either partially or completely block DA mediated behavior. For example, SCH 23390 (a selective D₁ receptor antagonist) inhibits grooming induced by the selective D₁ agonist SKF 38393; whereas, sulpiride (a

selective D₂ receptor antagonist) attenuates NPA-induced locomotor activity, rearing, and sniffing (Arnt, 1987; Clark & White, 1987). Interestingly, D₁ receptor blockade also antagonizes the enhanced locomotor activity, rearing, and sniffing induced by D₂ agonists (Arnt, 1987; Longoni et al., 1987). Additionally, the D₁ agonist SKF 38393 intensifies or enhances some behaviors induced by the D₂ agonist quinpirole (Mashurano & Waddington, 1989; Robertson & Robertson, 1986). This DA receptor synergism suggests that either a tonic level of D₁ activity is required for the expression of D₂-mediated behaviors, or the D₂ system modulates the expression of behaviors initiated by the D₁ system (Braun & Chase, 1986; McDougall et al., 1990; Molloy & Waddington, 1985; Robertson & Robertson, 1986).

The ability of DA antagonists to attenuate or block the effects of DA agonists has been discussed; however, DA antagonists are also capable of eliciting their own behavioral effects. DA receptor antagonists produce catalepsy in rats. For example, SCH 23390 and YM-09151 (selective D₁ and D₂ antagonists, respectively) produce a dose-dependant increase in catalepsy in adult rats (Christensen, Arnt, Hyttel, Larsen, & Svendsen, 1984; Terai, Usuda, Kuroiwa, Noshiro, & Maneno, 1983; Wanibuchi & Usuda, 1990). Furthermore, antagonists that block *both* D₁ and D₂ receptors (e.g., haloperidol and

flupenthixol) most potently induce catalepsy (Verma & Kulkarni, 1992; Wanibuchi & Usuda, 1990).

Behavioral effects of dopamine agonists and antagonists in preweanling rats. In general, preweanling rats exhibit adult-like behavior patterns when treated with direct and indirect DA agonists. Like adult rats, preweanling rats display increased rearing and locomotor activity after acute treatment with the indirect DA agonists amphetamine and cocaine (Barrett, Caza, Spear, & Spear, 1982; Campbell, Lytle, & Fibiger, 1969; Shalaby & Spear, 1980). Similarly, nonselective direct DA agonists (e.g., apomorphine and NPA) enhance locomotor activity and sniffing in both the preweanling and adult rat (Arnt, 1987; Mestlin & McDougall, 1993; Shalaby & Spear, 1980). Preweanling rats, in general, also display adult-like behavioral responses after treatment with selective DA agonists. For example, SKF 38393 (a selective D₁ agonist) increases locomotor activity in both 3- and 21-day-old rats, with increased sniffing evident in slightly older rats. Although the complete D₂ agonist behavioral profile (i.e., locomotor activity, rearing, and sniffing) is not observed until at least 21 days of age, quinpirole (a selective D₂ agonist) enhances locomotor activity at all ages (Eilam & Szechtman, 1989; Moody & Spear, 1992; Walters & Howard, 1990). Occasionally, DA agonists will produce

some age-dependant behavioral effects, however they are often due to the maturation of motoric ability and not because of qualitative changes in dopaminergic functioning.

As with adult rats, the D₁ and D₂ receptors of preweanling rats interact when mediating some behaviors, however the specific behaviors showing synergism often vary between preweanling and adult rats. For example, co-administration of D₁ and D₂ agonists (i.e., SKF 38393 and quinpirole) in 10-day-old rats induces a significant decrease in mouthing; whereas, 21-day-old rats show stereotyped licking after combined agonist treatment (Moody & Spear, 1992). Similarly, 3- and 10-day-old rats exhibit synergistic increases in DA agonist-induced wall climbing and locomotor activity, while they fail to show stereotyped oral movements. Therefore, the D₁ and D₂ receptors of preweanling rats appear to interact when mediating some behaviors, however, the specific behaviors showing D₁/D₂ synergism vary according to age.

Studies using DA antagonists also suggest that the DA receptor systems of young and adult rats are generally similar. For example, the quinpirole-induced locomotor activity of preweanling rats is antagonized by either SCH 23390 or sulpiride (D₁ and D₂ antagonists, respectively), while SKF 38393-induced grooming is blocked by SCH 23390 (McDougall et al., 1990). In

addition, selective D₁ and D₂ antagonists induce adult-like catalepsy in the preweanling rat, with the most intense catalepsy being induced by joint D₁/D₂ antagonist treatment (Baez, Burt, Granneman, & Shanklin, 1979; Fitzgerald & Hannigan, 1989).

To summarize, preweanling and adult rats display similar patterns of behavioral responding after DA agonist and antagonist treatment. In general, DA receptor activation elicits locomotor activity and rearing, with more stereotyped behaviors, such as sniffing, chewing, and biting, occurring when higher doses of a DA agonist are used. Furthermore, DA antagonists produce a dose-dependant decline in DA mediated behaviors, with catalepsy occurring at higher doses (Arnt, 1987; Baez et al., 1979; Clark & White, 1987).

Opiate Systems

Opioid Receptors. Opioid receptors have been classified into three structurally distinct receptor subtypes: mu, delta, and kappa (Lord, Waterfield, Hughes, & Kosterlitz, 1977; Martin, Eades, Thompson, Huppler, & Gilbert, 1976). Mu and delta receptors have been differentiated according to pharmacological action (Gilbert & Martin, 1976; Martin et al., 1976; Wollemann, Benyhe, & Simon, 1993). For example, receptor binding studies have revealed that morphine possesses high

affinity for mu receptors; whereas, delta receptors are activated by enkephalins (Brady & Holtzman, 1981; Wollemann et al., 1993). Pharmacological studies have shown that kappa receptors are different from mu and delta receptors, and they have been divided into three distinct subclasses: kappa₁, kappa₂, and kappa₃ receptors (Wollemann et al., 1993). More specifically, kappa₁ sites have a high affinity for benzeneacetamide compounds (like U-50,488); whereas, kappa₂ and kappa₃ sites display little affinity for U-50,488 (Wollemann et al., 1993).

The various opiate receptor subtypes can also be distinguished by their behavioral effects. It is known that mu agonists (e.g., DAGO and morphine) are involved in pain modulation and activate reward processes; whereas, kappa agonists (e.g., tifluadom, bremazocine, and U-50,488) reduce the spontaneous behavior of rats, and appear to have aversive effects when tested in the self-administration paradigm (Di Chiara & Imperato, 1988; Mucha & Herz, 1985; Yaksh, 1986). Evidence suggests that opiate receptor activation plays a largely modulatory role in behavior. In general, opiate receptor agonists inhibit the release of other neurotransmitters. Mu and delta agonists inhibit release of cortical noradrenaline and striatal acetylcholine; whereas, kappa receptor activation

inhibits dopamine release in the striatum and cortex (Wollemann et al., 1993).

Behavioral effects of mu and delta opioid agonists and antagonists in adult and preweanling rats. In general, mu receptor activation modulates pain and reward system functioning; however, the unlearned motor behaviors are affected as well. In adult rats, increased locomotor activity occurs after low doses of morphine (1-2 mg/kg); whereas, higher doses (5-20 mg/kg) induce behavioral depression, including reduced locomotor activity and catalepsy (Brady & Holtzman, 1981; Fog, 1972). In contrast, morphine and DAGO (mu agonists) have only cataleptic and sedative effects in the preweanling rat (Bolanos et al., 1995; Caza & Spear, 1982; Jackson & Kitchen, 1989; Katz, 1984).

Behavioral responses to delta agonists also vary across ontogeny. Treatment with low doses of DPDPE (a delta agonist) increases the locomotor activity, rearing, and sniffing of adult rats; whereas, higher doses induce behavioral depression (Cowan, Rance, & Blackburn, 1986; Cowan, Zhu, & Porreca, 1985). In contrast, DPDPE does not affect the behaviors of preweanling rats (Jackson & Kitchen, 1989). In summary, agonists at the mu and delta opiate receptor have pronounced age-dependent behavioral actions in preweanling and adult rats.

Behavioral effects of kappa opioid agonists and antagonists in adult rats. Kappa agonists induce conditioned place aversions and taste aversions in adult animals (Bechara & Van der Kooy, 1987; Mucha & Herz, 1985). The aversive properties of U-50,488 are mediated at both the peripheral and central level, with the central effects possibly resulting from decreased DA release within the nucleus accumbens (Bals-Kubic, Herz, & Shippenberg, 1989; Bechara & Van der Kooy, 1987; Di Chiara & Imperato, 1988). Kappa receptor activation also affects unlearned behaviors. In both rats and mice, decreased locomotor activity follows treatment with U-50,488, tifluadom, and berrmazocine (Castellano & Pavone, 1987; Crawford, McDougall, Bolanos, Hall, & Berger, 1995; Di Chiara & Imperato, 1988; Privette & Terrian, 1995; Von Voightlander et al., 1983). Interestingly, kappa agonists elicit a dose-dependant behavioral depression similar to that mediated by DA antagonists. For example, U-50,488 (5 or 10 mg/kg, s.c.) attenuates the locomotor activity of adult rats; whereas, catalepsy follows higher doses of the kappa agonist (Jackson & Cooper, 1985; Ukai & Kameyama, 1985; Von Voightlander et al., 1983).

Further evidence for the role of the kappa receptor in behavior is provided by studies using the selective kappa antagonists binaltorphimine and nor-

binaltorphimine (nor-BNI). Although these compounds have only recently been developed, it has been shown that U-50,488-induced catalepsy and antinociception are blocked by nor-BNI (Jones & Holtzman, 1992). The later results are important, because they clearly indicate that U-50,488 is affecting behavior by acting at the kappa receptor. In summary, the kappa opioid systems ability to decrease DA release inhibits unlearned activity, and the mediation of conditioned place aversion and taste aversion in adult rats (Bechara & Van der Kooy, 1987; Carr, Bak, Simon, & Portoghese, 1989; Crawford et al., 1995).

Behavioral effects of kappa opioid agonists and antagonists in preweanling rats. In direct contrast to adults, the kappa agonist U-50,488 elicits a dose-dependent increase in the locomotor activity of the preweanling rat (Bolanos et al., 1995; Jackson & Kitchen, 1989; Kehoe & Boylan, 1994). Furthermore, kappa agonists, like DA agonists, elicit age- and dose-dependant behavioral responding in the fetal and preweanling rat. For example, U-50,488 promotes dose-dependant increases in the activity of the fetal rat by as early as day 21 of gestation (Smotherman, Moody, Spear, & Robinson, 1993). In 5- and 10-day-old rats U-50,488 (0.1 and 1.0 mg/kg) preferentially enhances locomotor activity and wall-climbing; whereas, wall-

climbing predominates in the 20-day-old (Jackson & Kitchen, 1989). Dose is an important constraint on U-50,488's effects, as 10 mg/kg U-50,488 initially sedates the 20-day-old rat, with wall-climbing and locomotor activity emerging as the drug wears off (Birch & Hayes, 1987; Jackson & Kitchen, 1989).

The paradoxical effects of the kappa agonists in the preweanling rat, however, appear to be confined to increased locomotor activity. Similar to adult rats, kappa opiates produce an analgesic effect in preweanling rats (Barr, 1992; Barr, Paredes, Erickson, & Zukin, 1986). Furthermore, 3- and 7-day-old rats given U-50,488 display conditioned place aversions like adult rats (Barr, Wang, & Carden, 1994). These findings indicate that the analgesic and aversive properties of the kappa agonists are similar in adult and preweanling rats. Moreover, these results suggest that U-50,488's locomotor activating effects in the preweanling rat are not caused by the aversive effects of this drug (Barr et al., 1994; Carden, Barr, & Hofer, 1991).

The role of kappa receptors in mediating the behavioral responses of preweanling rats was confirmed using nor-BNI (a kappa antagonist). For example, U-50,488-induced analgesia and locomotor activity are blocked by nor-BNI (Jackson & Kitchen, 1989; Kehoe & Boylan, 1994). In contrast, selective mu or delta

antagonists (e.g., M8008 and ICI 174,864, respectively) do not affect the activity enhancing actions of U-50,488 in the preweanling rat (Birch & Hayes, 1987). Interestingly, treatment with SCH 23390 (a selective D₁ receptor antagonist) fails to inhibit the fetal motor behavior produced by U-50,488 (Smotherman et al., 1993). However, the kappa antagonist nor-BNI effectively blocks both U-50,488- and SKF 38393-induced motor activity in the fetal rat (Smotherman et al., 1993). This suggests that fetal motor behaviors are initially mediated by the kappa receptor, with the DA system only modulating kappa functioning (Robinson, Moody, Spear, & Smotherman, 1993; Smotherman et al., 1993). This DA-to-opioid interaction differs from the typical opioid-to-DA pattern reported in adult studies (DeVries, Hogenboom, Mulder, & Schoffelman, 1990; Mulder, Wardeh, Hogenboom, & Frankhuyzen, 1984; Schoffelman et al., 1988). This may reflect differences in experimental methodology, or may indicate ontogenetic changes in the relationship between the kappa opioid and DA systems (Smotherman et al., 1993). If the latter is correct, developmental differences could be mediated by changes in the distribution of kappa opioid receptors within the CNS (Smotherman et al., 1993).

In summary, although the behavioral responses elicited by kappa opioid agonists in adult rats have

been well documented, there has been relatively little examination of the behavioral effects of these drugs in the preweanling rat. Even so, kappa agonists do appear to produce pronounced age-dependant effects in the preweanling and adult rat. Kappa agonists inhibit behavioral responding in the adult; whereas, these same drugs enhance behavioral activity, including locomotion and wall climbing, in the preweanling rat (Bolanos et al., 1995; Jackson & Kitchen, 1989; Locke & Holtzman, 1986; Smotherman et al., 1993). Importantly, the pattern of these behavioral responses provides further evidence that the kappa opiate and DA systems interact to mediate behavior.

Dopamine Pathways

The dopaminergic system is highly organized topographically. On the basis of their efferent projections, dopaminergic cell groups have been broadly classified into two groups: (1) the mesolimbic-mesocortical pathway and (2) the nigrostriatal pathway (Cooper et al., 1991; Role & Kelly, 1991).

Mesolimbic-mesocortical pathway. DA fiber projections making up the mesolimbic-mesocortical pathway have their cell bodies in the ventral tegmental area (VTA). The cells of the mesolimbic component of this pathway project from the VTA to the limbic system, primarily the nucleus accumbens (Cooper et al., 1991;

Koob, 1992). Recently, an opioid-DA model has been proposed to explain the interactions between the opiate and DA systems (see Figure 1) (Di Chiara & North, 1992; Spanagel, Herz, & Shippenberg, 1992). Two opposing opioid systems, located in different parts of the mesolimbic system, modulate DA neurons projecting to the nucleus accumbens. Mu receptors located on GABA inhibitory neurons in the VTA disinhibit DA neurons projecting to the nucleus accumbens. Thus, the mu-induced reduction of GABA-mediated inhibition facilitates DA release in the nucleus accumbens. In contrast, kappa receptors, located on DA terminals in the nucleus accumbens, inhibit DA release. The opposing actions of the two opioid systems assist in the maintenance of basal DA release in the nucleus accumbens (Di Chiara & North, 1992; Spanagel et al., 1992). This model, and the evidence supporting it, suggests that the kappa opioid system plays an important role in modulating DA input, particularly in the nucleus accumbens.

Behavioral evidence generally supports this kappa/DA model. For example, microinjections of *d*-amphetamine into the nucleus accumbens produces locomotor activity in adult rats (Colle & Wise, 1991). Further, injections of NPA into the nucleus accumbens results in a dose-dependant increase in oral behaviors

and sniffing (Bordi et al., 1989). Although microinjection studies using kappa agonists have not yet been reported, systemic treatment with U-50,488 presumably depresses the locomotor activity of adult rats by decreasing DA release in the nucleus accumbens (Crawford et al., 1995; Di Chiara & Imperato, 1988). Consistent with this, kappa agonists reduce, and kappa antagonists increase, DA release in the accumbens (Di Chiara & Imperato, 1988; Spanagel et al., 1992).

Nigrostriatal pathway. The nigrostriatal pathway originates in the substantia nigra and projects primarily to the striatum, composed of the putamen and caudate nucleus (Nolte, 1981). The substantia nigra, which lies in the midbrain, has two zones: the pars reticulata and the pars compacta. Receptor binding studies indicate that large amounts of opiate receptors (mu and kappa) are present in the pars compacta, with low levels present in the pars reticulata (Matsumoto, Brinsfield, Patrick, & Walker, 1988; Merchenthaler, Maderdrut, Altschuler, & Petrusz, 1986). Evidence suggests that kappa opioid agonists have dual opposing effects on activity: a motor activating effect mediated by nondopaminergic pars reticulata cells and a motor inhibitory effect mediated through the pars compacta DA cells (Matsumoto et al., 1988). For example, treatment with U-50,488 reduces locomotor activity by decreasing

the firing rate of dopamine cells in the substantia nigra (Walker, Thompson, Frascella, & Frederick, 1987) and by decreasing the release of dopamine in the striatum (Imperato & Di Chiara, 1985).

Microinjections of DA agonists directly into the striatum produce a wide range of behavioral responses. Episodes of DA-mediated locomotor activity, rearing, and grooming follow injections of low doses of NPA into the striatum (Bordi et al., 1989). Furthermore, injections of NPA or apomorphine into the striatum also enhances stereotyped sniffing and oral behaviors. At doses from 5.0-20 μ g a linear increase in both behaviors is evident; whereas, at higher doses (40 μ g) the increased receptor occupancy results in stereotyped behavior, comprised of enhanced oral behaviors accompanied by a decrease in sniffing (Bordi et al., 1989; Scheel-Kruger & Arnt, 1985). Furthermore, microinjections of U-50,488 into the substantia nigra pars reticulata elicits long-duration rotational behavior (Matsumoto et al., 1988). These findings suggest that kappa opiate receptors located in the nigrostriatal pathway are involved in the regulation of DA-mediated motor function. This evidence indicates that opiate receptor agonists elicit behavioral responses by activating both the mesolimbic-mesocortical and nigrostriatal pathways (Matsumoto et al., 1988).

In summary, DA pathways are both topographically and behaviorally dissociable. The mesolimbic-mesocortical pathway projects from the ventral tegmental area to limbic and cortical regions and appears to be necessary for a number of DA-mediated behaviors. The nigrostriatal pathway projects from the substantia nigra to several striatal regions and is most critically involved in mediating stereotyped behavior. Evidence shows that kappa opiate receptors are present and active in both pathways, affecting behavior by modulating DA release.

Fos Immunohistochemistry. Fos immunohistochemistry is a new technique which appears ideally suited for determining the precise location of neuronal activation. Various DA-acting drugs elicit the transient expression of Fos, the product of the early response gene *c-fos* (Dragunow, Robertson, Faull, Robertson, & Jansen, 1990; Graybiel, Moratalla, & Robertson, 1990). Therefore, Fos expression reflects neuronal activity in the CNS (Miller, 1990; Sagar, Sharp, & Curran, 1988). Following treatment with amphetamine, enhanced locomotor activity is accompanied by extensive *c-fos* induction in the medial two-thirds of the striatum, as well as the nucleus accumbens, olfactory tubercles, and a variety of other brain regions (Bullitt, 1990; Graybiel et al., 1990; Sharp, Gonazales, Sharp, & Sagar, 1989; Snyder-

Keller, 1991; Torres & Rivier, 1994). Likewise, cocaine produces large increases in striatal *c-fos* expression, which is potently blocked by the selective D₁ receptor antagonist SCH 23390 (Carney, Tolliver, Carney, & Kindy, 1991; Dragunow et al., 1990). Utilization of Fos immunohistochemistry following administration of the kappa agonist U-50,488 should elucidate the specific brain areas involved in the opioid activation of behavior.

Kappa/Dopamine Interactions

Behavioral and pharmacological evidence suggests that kappa receptors may affect behavior by modulating the functioning of DA neurons. Consistent with this, Crawford et al. (1995) have shown that the kappa opioid agonist U-50,488 antagonizes cocaine-induced locomotor activity in adult rats. This indicates that kappa neurons modulate those DA systems involved in unlearned activity. In addition, cocaine-induced Fos immunoreactivity in the adult rat is blocked by U-50,488 pretreatment, further indicating that the DA and kappa opioid systems interact (Crawford et al., 1995).

In summary, behavioral studies indicate that there is a substantial ontogenetic change in the way that the kappa and DA systems interact to mediate behavior. More specifically, U-50,488 *increases* the locomotor activity of preweanling rats, while *decreasing* the locomotor

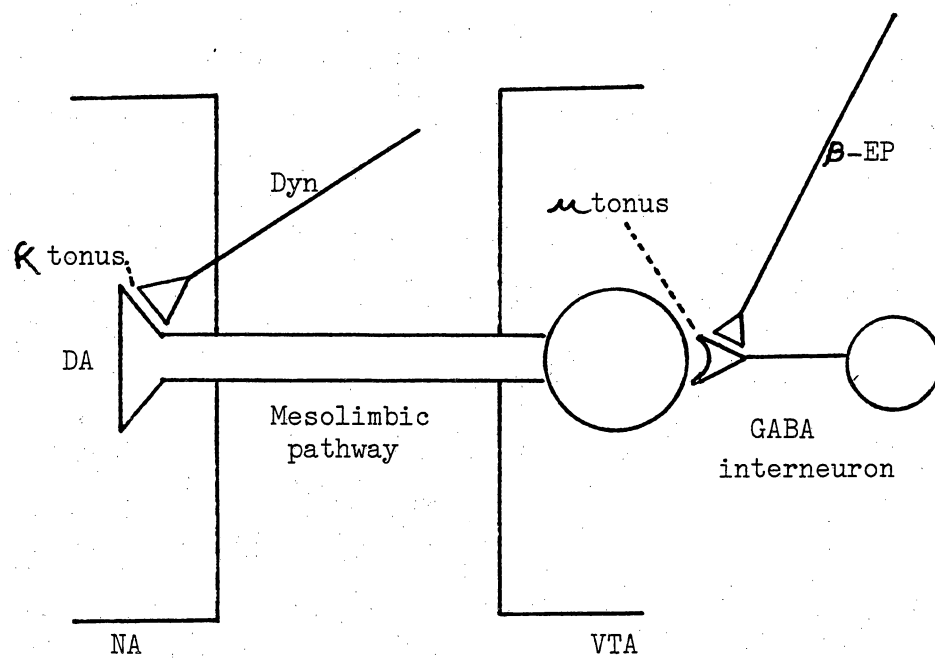
activity of adult rats (Bolanos et al., 1995; Jackson & Kitchen, 1989; Kehoe & Boylan, 1994; Smotherman et al., 1993). In adults, the U-50,488-induced decrease in locomotor activity is presumably due to the modulation of DA functioning in the substantia nigra and/or nucleus accumbens; however, the neurobiological mechanisms underlying U-50,488's behavioral activating effects in preweanling rats is entirely unknown. Interestingly, the locomotor activating effects of kappa and DA agonists are similar in the preweanling rat, suggesting that kappa neurons may, in some way, modulate those DA systems involved with unlearned activity. The present study further examined this kappa/DA interaction in order to elucidate the mechanisms involved in the kappa-mediated behaviors of preweanling rats.

This project assessed the possible interaction between the DA and kappa opioid systems in the preweanling rat. A total of six hypotheses were proposed: (1) I proposed that the kappa agonist U-50,488 would decrease locomotor activity in the adult rat: a result that has been reported previously (Crawford et al., 1995); (2) As previously shown, U-50,488 was expected to increase the locomotor activity of the preweanling rat (Bolanos et al., 1995; Jackson & Kitchen, 1989; Kehoe & Boylan, 1994); (3) U-50,488 was expected to potentiate amphetamine- and NPA-induced

locomotor activity and sniffing. This would indicate that U-50,488 and the DA agonists are modulating behavior by affecting the same neuroanatomical pathways; (4) Flupenthixol (a nonselective DA receptor antagonist) was predicted to reduce U-50,488-mediated locomotor activity. This would indicate that the behaviors elicited by this agonist are ultimately DA mediated; (5) Amphetamine and NPA would induce regional expression of Fos immunoreactivity in the preweanling rat: an observation observed in adult rats, but not tested in preweanling rats (Robertson, Peterson, Murphy, & Robertson, 1989; Snyder-Keller, 1991); (6) U-50,488-, amphetamine-, and NPA-mediated Fos immunoreactivity was predicted to occur in the same brain regions. This would pinpoint those brain areas involved in unlearned activity; moreover, it would indicate that the kappa and DA agonists are affecting the same pathways.

FIGURE CAPTION

Figure 1. Proposed model for the modulation of DA neurons by opposing tonically active endogenous opioid systems. In the ventral tegmental area a tonically active mu opioid system stimulates the DA neurons via disinhibition of the GABA-containing interneuron. In the nucleus accumbens a tonically inhibitory kappa opioid system suppresses the release of DA by A10 neurons. The action of both opioid systems is necessary for the maintenance of basal DA release.



General Method

Subjects

Subjects were 248 male and 248 female Sprague-Dawley rats (Harlan). Litters were culled to 8 to 10 rat pups at three days of age. Rats were tested when either 17 or 80 days of age. The 17-day-olds were housed with the dam prior to behavioral testing. The 80-day-old rats were individually housed until the test day. Assignment of male and female rat pups was counterbalanced, with no more than one rat from each litter being placed into a particular group. The colony room was maintained at 23-25°C and kept under a 14:10/L:D cycle. Behavioral testing was conducted during the light phase of the cycle.

Apparatus

Behavioral testing was done in grey plywood chambers (30 x 30 x 42 cm), with floors divided by lines into four equal quadrants.

Evaluation of behavioral testing

A single line-cross was scored when a rat's front two paws completely crossed one of the lines dividing the floor of the testing chamber. Line-crossing was measured continuously across the testing session, whereas the occurrence of stereotyped (head-down) sniffing was assessed every 20 s using a time sampling procedure.

Drugs

The drugs used were *d*-amphetamine sulfate (Sigma Chemicals, St. Louis, Mo.), *R*-propylnorapomorphine (NPA), U-50,488, and flupenthixol (Research Biochemicals, Natick, Mass). All drugs were mixed in saline at a volume of 5 ml/kg. U-50,488 was injected subcutaneously (s.c.), whereas all other drugs were injected intraperitoneally (i.p.).

Statistics

Analyses of variance (ANOVAs) were used for the statistical analysis of both the behavioral and immunohistochemistry data. For the locomotor activity data repeated measures ANOVAs were performed across six 10-min time blocks; whereas, the sniffing data was analyzed across two 30-min time blocks for the initial four experiments. For the remaining experiments, sniffing data was analyzed using repeated measures ANOVAs across six 10-min time blocks.

Data from the Fos immunohistochemistry experiment were analyzed using two-way ANOVAs (for each brain region). When appropriate, Tukey tests ($P < 0.05$) were used for making post hoc comparisons. U-50,488 has been shown to increase the locomotor activity of the preweanling rat (Bolanos et al., 1995). Therefore, it was predicted that U-50,488 would enhance Fos expression in the 17-day-old rat, but not the adult rat. To

analyze the effects of U-50,488 on Fos expression *t*-tests ($P < 0.05$) were used to compare U-50,488- and saline-treated rats.

Experiment 1a

The Behavioral Effects of U-50,488, a Kappa Opioid Agonist, in 17-Day-Old Rats

Method

Subjects. Thirty-two male and female 17-day-old Sprague-Dawley albino rats (Harlan) were used.

Procedure. Four groups of 17-day-old rats ($n = 8$) received an acute injection of U-50,488 (0, 2, 5, or 10 mg/kg) immediately prior to the behavioral testing session. Line-crosses and stereotyped (head-down) sniffing were assessed during a 60-min testing session by an observer blind to treatment conditions.

Results

Line-crosses. Overall, 17-day-old rats treated with U-50,488 (2.0, 5.0, 10.0 mg/kg) had significantly more line-crosses than saline controls (see Figure 2) [condition main effect, $F(3,28)=59.16$, $P < 0.001$; and Tukey tests, $P < 0.05$]. The total number of line-crosses decreased across testing [time main effect, $F(5,140)=8.68$, $P < 0.001$; and Tukey tests, $P < 0.05$]. During the initial testing block, rats injected with 10.0 mg/kg U-50,488 had significantly more line-crosses than rats given 2.0 mg/kg or 5.0 mg/kg U-50,488

[Condition X Time interaction, $F(15,140)=3.82$, $P < 0.001$; and Tukey tests, $P < 0.05$]. However, for the remainder of testing there were no significant differences between U-50,488-treated rats.

Stereotyped sniffing. There were no significant differences between 17-day-old rats injected with either U-50,488 or saline (see Table 1).

FIGURE CAPTION

Figure 2. Mean number of line-crosses during the 60-min behavioral testing session. The 17-day-old rats ($n = 8$) were injected with U-50,488 (2.0, 5.0, or 10.0 mg/kg, s.c.) or saline immediately prior to behavioral testing.

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Line—Crosses

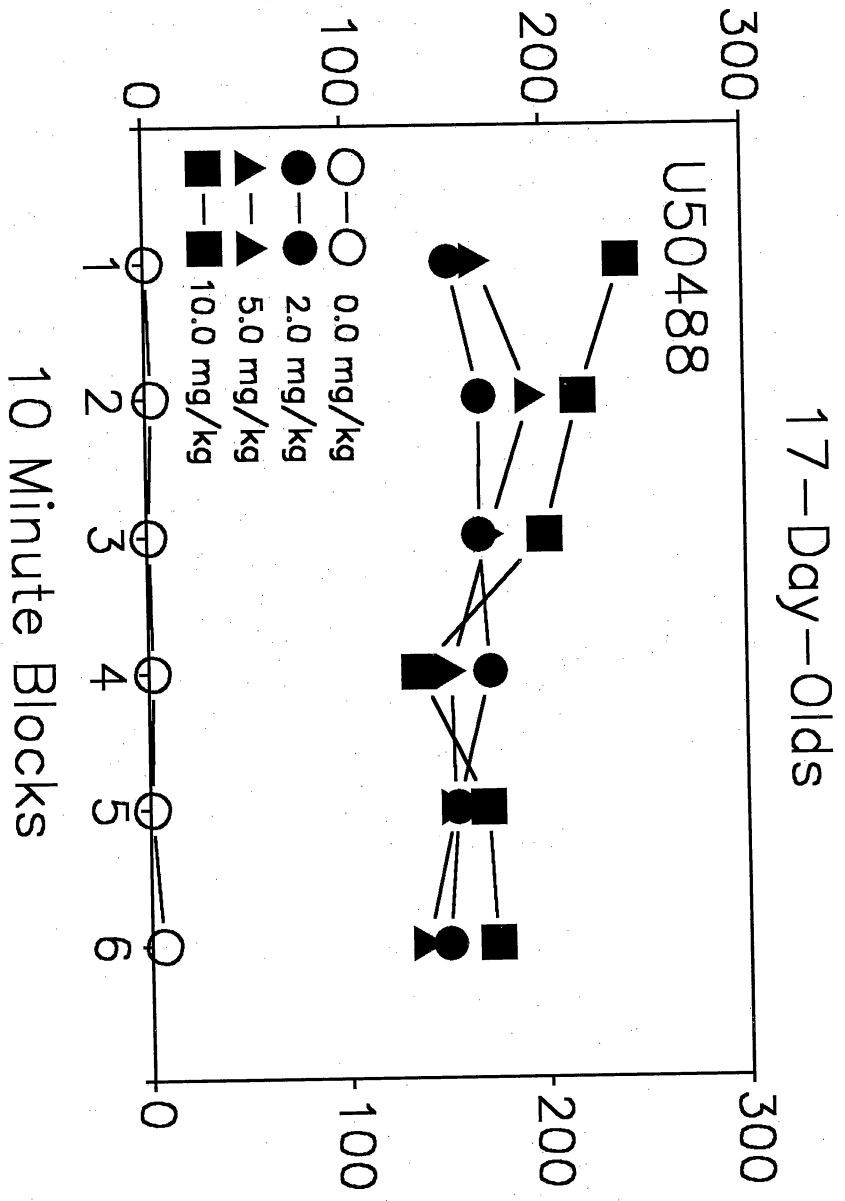


Table 1. Mean stereotyped sniffing counts (\pm SEM) of 17-day-old rats given U-50,488 (0.0--10.0 mg/kg). Behavioral testing occurred immediately after U-50,488 treatment and was recorded for 60 min (divided into two 30-min testing periods).

U-50,488	Stereotyped Sniffing Counts	
	First 30 Min	Second 30 Min
0.0 mg/kg	0.75 \pm 0.49	0.25 \pm 0.25
2.0 mg/kg	4.63 \pm 0.94	2.25 \pm 0.79
5.0 mg/kg	6.25 \pm 5.97	2.63 \pm 2.63
10.0 mg/kg	0.88 \pm 0.49	1.50 \pm 0.59

Experiment 1b

The Behavioral Effects of Amphetamine, an Indirect DA Agonist, in 17-Day-Old Rats

Method

Subjects. Thirty-two male and female 17-day-old Sprague-Dawley albino rats (Harlan) were used.

Procedure. Four groups of 17-day-old rats ($n = 8$) received an acute injection of amphetamine (0, 1, 2.5, or 5 mg/kg) immediately prior to behavioral testing. Line-crosses and stereotyped (head-down) sniffing were assessed during a 60-min testing session by an observer blind to treatment conditions.

Results

Line-crosses. Overall, rats given amphetamine had significantly more line-crosses than rats receiving saline (see Figure 3) [condition main effect, $F(3,28)=3.90$, $P<0.05$; and Tukey tests, $P<0.05$]. The total number of line-crosses decreased across testing [time main effect, $F(5,140)=19.01$, $P<0.001$; and Tukey tests, $P<0.05$]. Across the initial two testing blocks rats receiving amphetamine had significantly more line-crosses than the saline controls (see Figure 3) [Condition X Time interaction, $F(15,140)=2.17$, $P<0.01$; and Tukey tests, $P<0.05$]. However, only rats receiving 1.0 mg/kg amphetamine had significantly more line-crosses than saline controls on each of the testing

blocks [Tukey tests, $P < 0.05$].

Stereotyped sniffing. Overall, rats given 5.0 mg/kg amphetamine had significantly more stereotyped sniffing counts than saline-treated rats (see Table 2) [condition effect, $F(3,28)=4.15$, $P < 0.05$; and Tukey tests, $P < 0.05$]. Rats receiving 1.0 and 2.5 mg/kg amphetamine did not differ from the saline controls.

FIGURE CAPTION

Figure 3. Mean number of line-crosses during the 60-min behavioral testing session. The 17-day-old rats ($n = 8$) were injected with amphetamine (1.0, 2.5, or 5.0 mg/kg, i.p.) or saline immediately prior to behavioral testing.

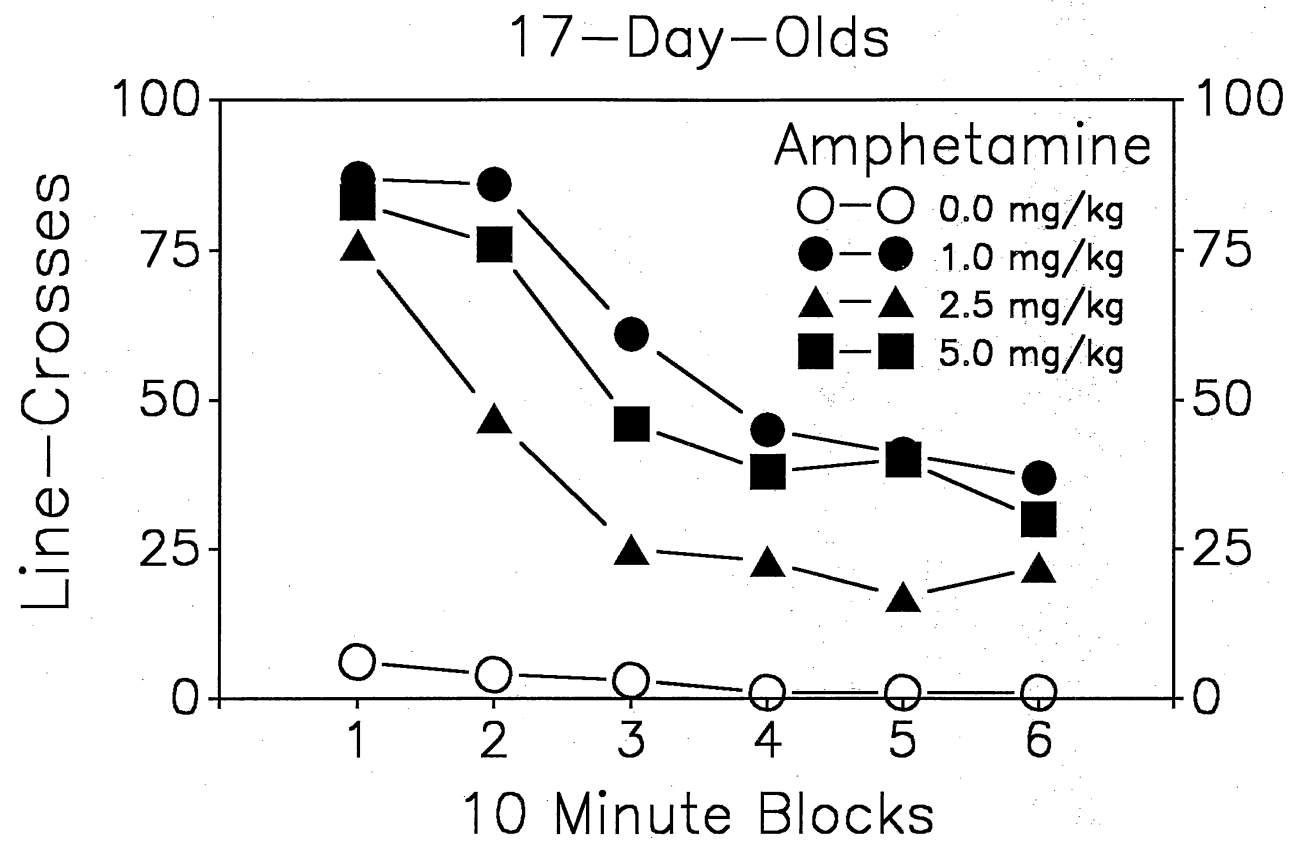


Table 2. Mean stereotyped sniffing counts (\pm SEM) of 17-day-old rats given amphetamine (0.0--5.0 mg/kg). Behavioral testing occurred immediately after treatment and was recorded for 60 min (divided into two 30-min testing periods).

Amphetamine	Stereotyped Sniffing Counts	
	First 30 Min	Second 30 Min
0.0 mg/kg	0.50 \pm 0.33	0.50 \pm 0.38
1.0 mg/kg	4.00 \pm 2.67	2.25 \pm 1.14
2.5 mg/kg	3.13 \pm 2.11	1.50 \pm 1.05
5.0 mg/kg	12.00 \pm 4.74 ^A	3.25 \pm 1.19 ^A

^ASignificant condition main effect indicating that amphetamine-treated rats were significantly different from the saline-treated rats, $P < 0.05$.

Experiment 1c

The Behavioral Effects of NPA, a Direct DA Agonist, in 17-Day-Old Rats

Method

Subjects. Thirty-two male and female 17-day-old Sprague-Dawley albino rats (Harlan) were used.

Procedure. Four groups of 17-day-old rats ($n = 8$) received an acute injection of NPA (0, 0.1, 0.3, or 1.0 mg/kg) immediately prior to behavioral testing. Line-crosses and stereotyped (head-down) sniffing were assessed during a 60-min testing session by an observer blind to treatment conditions.

Results

Line-crosses. Overall, all of the NPA-treated groups had significantly more line-crosses than the saline group (see Figure 4) [condition main effect, $F(3,28)=16.74$, $P<0.001$; and Tukey tests, $P<0.05$]. The total number of line-crosses decreased across testing [time main effect, $F(5,140)=32.96$, $P<0.001$; and Tukey tests, $P<0.05$]. Rats given 0.3 and 1.0 mg/kg NPA had significantly more line-crosses than the saline controls on all of the testing blocks [Condition X Time interaction, $F(15,140)=3.25$, $P<0.001$; and Tukey tests, $P<0.05$]. The rats receiving 0.1 mg/kg NPA only differed from the saline controls on the first two testing blocks [Tukey tests, $P<0.05$].

Stereotyped sniffing. Overall, 17-day-old rats injected with NPA had significantly more stereotyped sniffing counts than saline controls (see Table 3) [condition main effect, $F(3,28)=16.74$, $P<0.001$; and Tukey tests, $P<0.05$]. The differences between the NPA and saline groups were apparent on both test periods [Condition X Time interaction, $F(3,28)=3.41$, $P<0.05$; and Tukey tests, $P<0.05$]. During the second testing period, rats given 0.3 mg/kg NPA had significantly more sniffing counts than all other groups [Tukey tests, $P<0.05$].

FIGURE CAPTION

Figure 4. Mean number of line-crosses during the 60-min behavioral testing session. The 17-day-old rats ($n = 8$) were injected with NPA (0.1, 0.3, or 1.0 mg/kg, i.p.) or saline immediately prior to behavioral testing.

17-Day-Olds

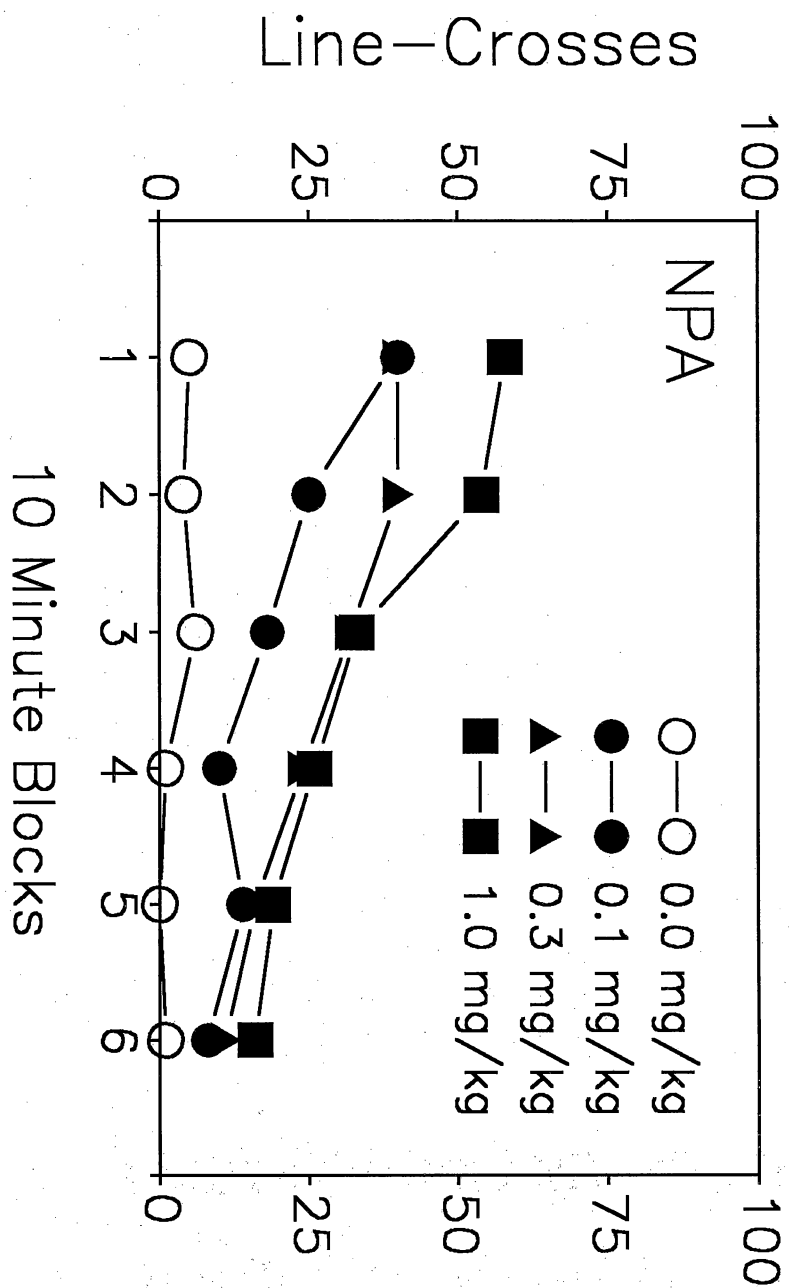


Table 3. Mean stereotyped sniffing counts (\pm SEM) of 17-day-old rats given NPA (0.0--1.0 mg/kg). Behavioral testing occurred immediately after NPA treatment and was recorded for 60 min (divided into two 30-min testing periods).

NPA	Stereotyped Sniffing Counts	
	First 30 Min	Second 30 Min
0.0 mg/kg	1.12 \pm 0.44	0.00 \pm 0.00
0.1 mg/kg	32.75 \pm 2.06 ^a	24.13 \pm 2.83 ^a
0.3 mg/kg	38.13 \pm 6.18 ^a	44.75 \pm 5.63 ^{a b}
1.0 mg/kg	38.50 \pm 5.45 ^a	23.75 \pm 5.03 ^a

^aSignificant Condition X Time interaction indicating that rats given NPA had significantly more stereotyped sniffing counts than saline-treated rats during both testing periods, $P < 0.05$.

^bSignificant Condition X Time interaction indicating that the 0.3 mg/kg NPA group was significantly different from all other groups during the final 30-min testing period, $P < 0.05$.

Experiment 1d

The Behavioral Effects of U-50,488, a Kappa Opioid Agonist, in 80-Day-Old Rats

Method

Subjects. Thirty-two male and female 80-day-old Sprague-Dawley albino rats (Harlan) were used.

Procedure. Four groups of 80-day-old rats ($n = 8$) received an acute injection of U-50,488 (0, 2, 5, or 10 mg/kg) immediately prior to behavioral testing. Line-crosses and stereotyped (head-down) sniffing were assessed during a 60-min testing session by an observer blind to treatment conditions.

Results

Line-crosses. Overall, rats given 2.0 mg/kg U-50,488 had significantly more line-crosses than rats receiving saline (see Figure 5) [condition main effect, $F(3,28)=3.63$, $P<0.05$; and Tukey tests, $P<0.05$]. There was a rapid decline in the total number of line-crosses across testing [time main effect, $F(5,140)=107.18$, $P<0.001$; and Tukey tests, $P<0.05$]. For example, from the first to second testing block, there was a significant decrease in line-crosses of rats given 10.0 mg/kg U-50,488 [Condition X Time interaction, $F(15,140)=3.26$, $P<0.001$; and Tukey tests, $P<0.05$]. Rats given 2.0 mg/kg U-50,488 had significantly more line-crosses than saline- and 10 mg/kg U-50,488-treated rats

during the second testing block [Tukey tests, $P < 0.05$]. There were no significant differences between U-50,488- and saline-treated rats during the fourth and fifth testing blocks. During the final testing block, rats given 10.0 mg/kg U-50,488 had significantly more line-crosses than saline-treated rats [Tukey tests, $P < 0.05$].

Stereotyped sniffing. Overall, the total number of sniffs declined from the first to the second testing period (see Table 4) [time main effect, $F(1,28)=75.81$, $P < 0.001$]. There were no significant differences in sniffing counts between saline- or U-50,488-treated rats.

FIGURE CAPTION

Figure 5. Mean number of line-crosses during the 60-min behavioral testing session. The 80-day-old rats ($n = 8$) were injected with U-50,488 (2.0, 5.0, or 10.0 mg/kg, s.c.) or saline immediately prior to behavioral testing.

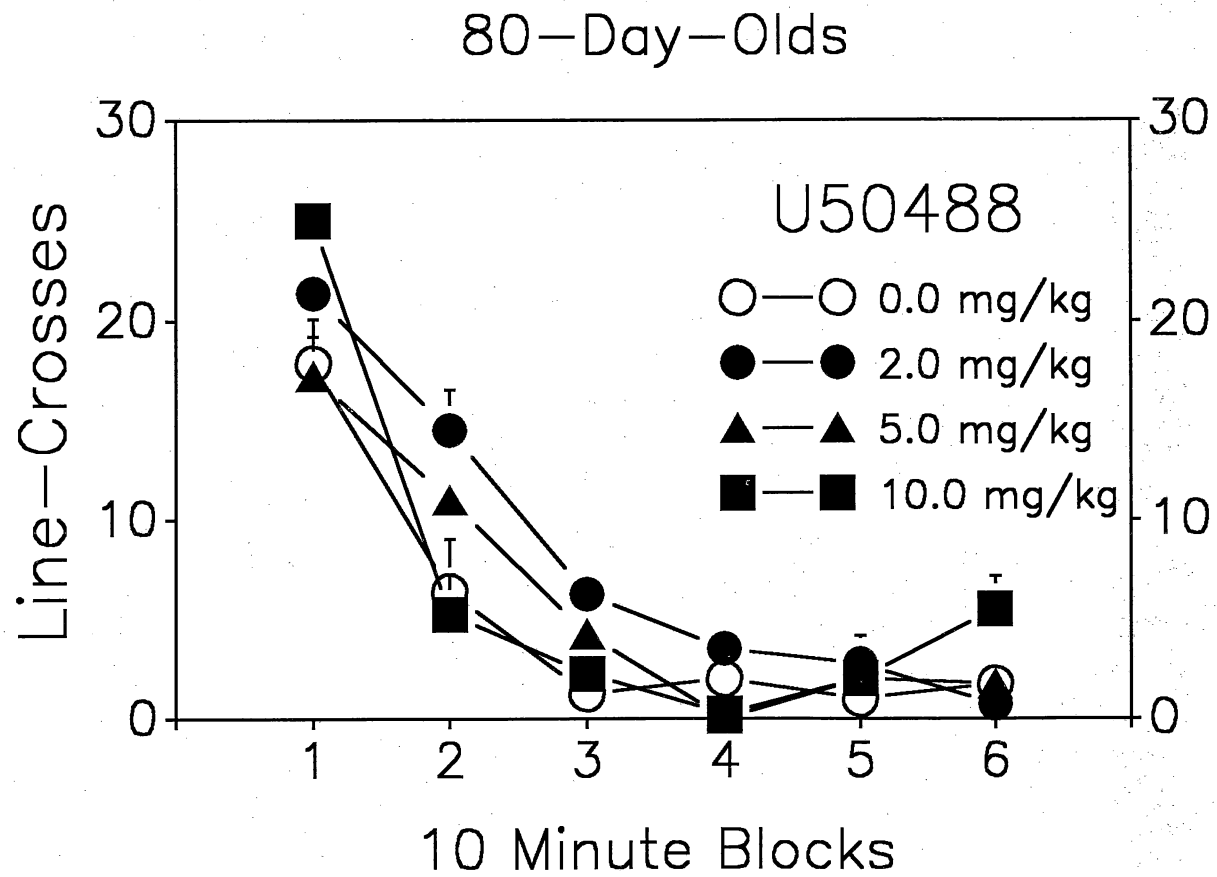


Table 4. Mean stereotyped sniffing counts (\pm SEM) of 80-day-old rats given U-50,488 (0.0--10.0 mg/kg). Behavioral testing occurred immediately after U-50,488 treatment and was recorded for 60 min (divided into two 30-min testing periods).

U-50,488	Stereotyped Sniffing Counts	
	First 30 Min	Second 30 Min
0.0 mg/kg	2.75 \pm 0.86	2.25 \pm 0.61 ^A
2.0 mg/kg	4.25 \pm 0.82	4.25 \pm 0.92 ^A
5.0 mg/kg	4.13 \pm 0.63	4.00 \pm 0.65 ^A
10.0 mg/kg	6.25 \pm 0.12	5.25 \pm 0.52 ^A

^ASignificant time main effect indicating that the total number of sniffing counts in the second 30-min testing period was significantly different from the first 30-min testing period, $P < 0.05$.

Discussion

Results showed that U-50,488-treatment dramatically increased the locomotor activity of 17-day-old rats. However, U-50,488 had no effect on the stereotyped sniffing of preweanling rats. In the 17-day-old rat 5.0 mg/kg U-50,488 produced a consistent level of locomotor activity across the testing session. In 80-day-old rats, 5.0 mg/kg U-50,488 produced a rapid decline in locomotor activity, while having no effect on stereotyped sniffing. Based on these results the 5.0 mg/kg dose of U-50,488 was used in subsequent experiments.

All doses of amphetamine and NPA increased the locomotor activity of 17-day-old rats. Importantly, high doses of a DA agonist can induce stereotypical behavior in the rat, often masking a drug's locomotor activating effects. Therefore, based on these results 2.5 mg/kg amphetamine and 1.0 mg/kg NPA were used for future experiments.

Experiment 2

The Effects of the DA Antagonist, Flupenthixol, on U-50,488-Induced Behaviors in 17-Day-Old Rats

This experiment was designed to determine whether U-50,488 works through a dopaminergic mechanism. More specifically, if flupenthixol blocked U-50,488's behavioral effects it would suggest that U-50,488 increases activity by enhancing DA functioning.

Method

Subjects. Forty-eight male and female 17-day-old Sprague-Dawley albino rats (Harlan) were used.

Procedure. Six groups of 17-day-old rat rats ($n = 8$) received an acute injection of the dopamine receptor antagonist flupenthixol (0, 0.025, 0.1, 0.4, or 0.8 mg/kg) 30 min prior to behavioral testing. The same rats then received U-50,488, NPA, or amphetamine immediately prior to a 60-min behavioral testing session.

Results

Line-crosses. Overall, rats pretreated with 0.4 or 0.8 mg/kg flupenthixol had significantly fewer line-crosses than saline-pretreated rats (see Figure 6) [condition main effect, $F(4,35)=37.43$, $P<0.001$; and Tukey tests, $P<0.05$]. Pretreatment with 0.8 mg/kg flupenthixol significantly reduced U-50,488-induced

locomotor activity on each of the testing blocks [Condition X Time interaction, $F(20,175)=4.48$, $P<0.001$; and Tukey tests, $P<0.05$]. Furthermore, pretreatment with 0.4 mg/kg flupenthixol attenuated U-50,488-induced locomotor activity across the last four testing blocks [Tukey tests, $P<0.05$]. Neither 0.025 or 0.1 mg/kg flupenthixol had any significant effects on U-50,488-induced locomotor activity.

Stereotyped sniffing. Overall, the total number of sniffing counts declined from the first to second 30-min testing period (see Table 5) [time main effect, $F(1,35)=15.31$, $P<0.05$]. However, there were no significant differences due to condition.

FIGURE CAPTION

Figure 6. Mean number of line-crosses during the 60-min behavioral testing session. The 17-day-old rats ($n = 8$) were injected with flupenthixol (0.025, 0.1, 0.4, or 0.8 mg/kg, i.p.) 60 min prior to behavioral testing. Rats were then given U-50,488 (5.0 mg/kg, s.c.), amphetamine (2.5 mg/kg, i.p.), NPA (1.0 mg/kg, i.p.) or saline immediately prior to testing.

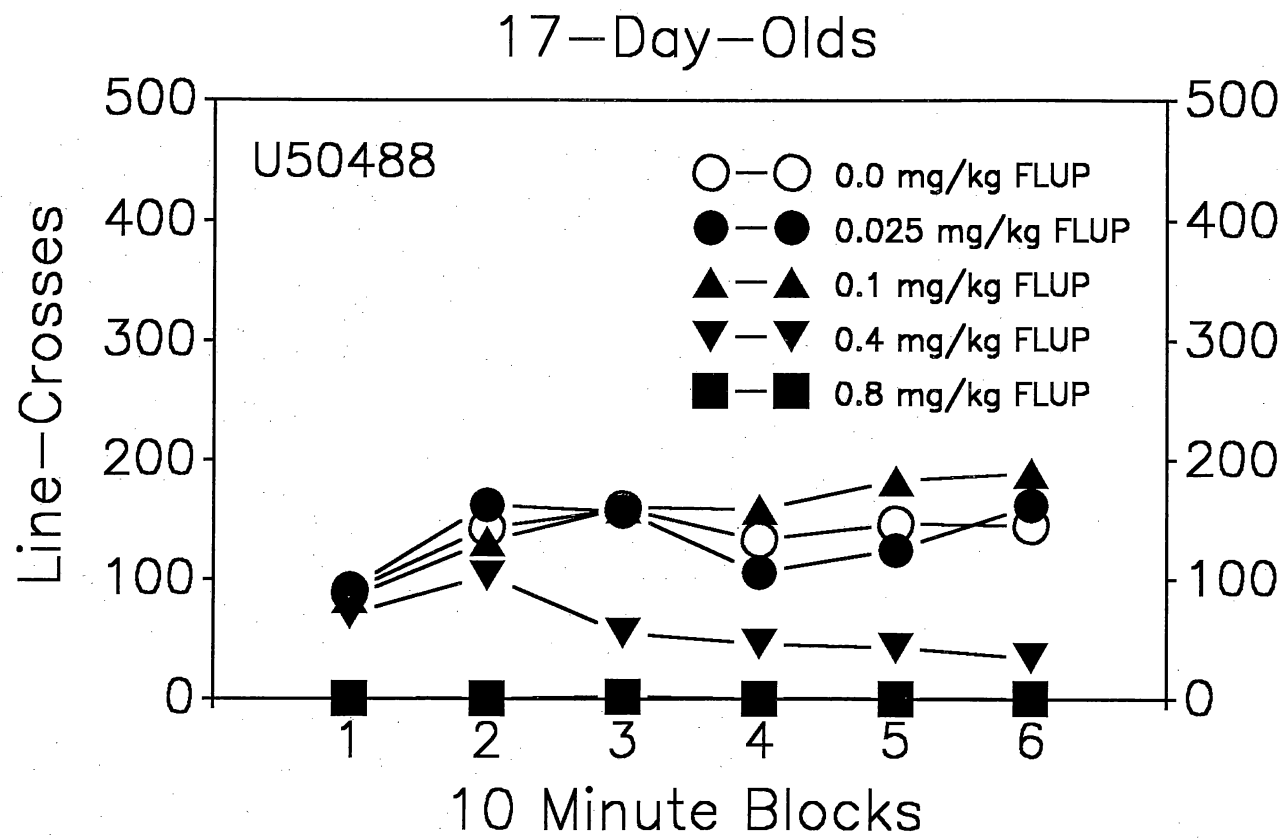


Table 5. Mean stereotyped sniffing counts (\pm SEM) of 17-day-old rats given U-50,488 (5.0 mg/kg) 30 min after treatment with flupenthixol (0.0--0.8 mg/kg). Behavioral testing occurred immediately after U-50,488 treatment and was recorded for 60 min (divided into two 30-min testing periods).

Flupenthixol	Stereotyped Sniffing Counts	
	First 30 Min	Second 30 Min
0.0 mg/kg	2.75 \pm 1.37	1.63 \pm 0.77 ^A
0.025 mg/kg	2.63 \pm 1.29	0.88 \pm 0.52 ^A
0.1 mg/kg	1.13 \pm 0.44	0.25 \pm 0.25 ^A
0.4 mg/kg	1.25 \pm 0.41	0.63 \pm 0.63 ^A
0.8 mg/kg	0.00 \pm 0.00	0.00 \pm 0.00 ^A

^ASignificant time main effect indicating that the total number of sniffing counts in the second 30-min testing period was significantly different from the the first 30-min testing period, $P < 0.05$.

Discussion

The goal of this experiment was to determine the effects of flupenthixol, a DA antagonist, on U-50-488-induced behaviors. Flupenthixol did produce a decline in U-50,488-induced behaviors in the 17-day-old rat. This decrease in activity suggests that U-50,488's behavioral effects may work via a dopaminergic mechanism. However, it is possible that flupenthixol may have decreased U-50,488-induced activity by a general depression of motoric functioning. Therefore, subsequent experiments were done to further examine the interaction between the kappa opioid and DA systems. To that end, rats were pretreated with the kappa receptor agonist U-50,488, then received either the indirect DA agonist amphetamine or the direct DA agonist NPA. If these drugs are working through a dopaminergic mechanism one would predict that U-50,488 pretreatment would potentiate DA agonist-induced activity.

Experiment 3a

The Effects of Amphetamine or NPA on U-50,488-Induced Behaviors in the 17-Day-Old Rat

The goal of the present experiment was to determine whether the kappa opioid and DA agonists activate behavior through the same mechanism. More specifically, if U-50,488 potentiated amphetamine's and/or NPA's locomotor activating effects it would suggest that these drugs are ultimately activating the same pathways.

Method

Subjects. Forty-eight male and female 17-day-old Sprague-Dawley albino rats (Harlan) were used.

Procedures. Six groups of 17-day-old rats ($n = 8$) received an acute injection of U-50,488 immediately prior to a 60-min behavioral testing session. Subjects then received an acute injection of amphetamine, NPA, or saline 30 min into the behavioral testing session. One hour after behavioral testing, rats were anesthetized with phenobarbital and rapidly perfused with 4% paraformaldehyde for later analysis of Fos immunoreactivity.

Results

Line-crosses. Overall, 17-day-old rats treated with U-50,488 had significantly more line-crosses than saline-treated rats across the initial three testing blocks (see left panel, Figure 7) [Pre X Time

interaction, $F(2,92)=24.35$, $P<0.001$; and Tukey tests, $P<0.05$]. Interestingly, across the final three testing blocks, NPA produced a progressive decline in U-50,488-induced line-crosses; whereas, amphetamine had no effect on U-50,488-induced activity (see upper graph, right panel, Figure 7) [Pre X Post X Time interaction, $F(4,84)=7.95$, $P<0.001$; and Tukey tests, $P<0.05$].

By itself, NPA significantly increased the line-crosses of saline-pretreated rats (lower graph, right panel, Figure 7); however, rats given both U-50,488 and NPA did not differ from rats given NPA alone [Pre X Post X Time interaction, $F(4,84)=7.95$, $P<0.001$; and Tukey tests, $P<0.05$]. Amphetamine alone also significantly increased the line-crosses of saline-pretreated rats (lower graph, right panel, Figure 7) [Tukey tests, $P<0.05$]. Rats given both U-50,488 and amphetamine had significantly more line-crosses than rats given amphetamine alone [Tukey tests, $P<0.05$] (right panel, Figure 7).

Stereotyped sniffing. There were no significant differences between saline- or U-50,488-pretreated rats during the initial 30-min testing period. Overall, U-50,488-treated rats had significantly fewer stereotyped sniffing counts than saline-pretreated rats during the final testing period (see Table 6) [pre main effect, $F(1,47)=4.42$, $P<0.05$]. This effect was entirely due to

NPA's actions however, as rats given NPA had significantly more stereotyped sniffing counts than the saline controls during the second 30-min testing period [post main effect, $F(2,47)=65.17$, $P<0.001$; and Tukey tests, $P<0.05$].

FIGURE CAPTION

Figure 7. Mean number of line-crosses during the 60-min behavioral testing session. The 17-day-old rats ($n = 8$) were injected with U-50,488 (5.0 mg/kg, s.c.) or saline immediately prior to behavioral testing. Rats were then injected with amphetamine (2.5 mg/kg, i.p.), NPA (1.0 mg/kg, i.p.) or saline 30 min into the testing session.

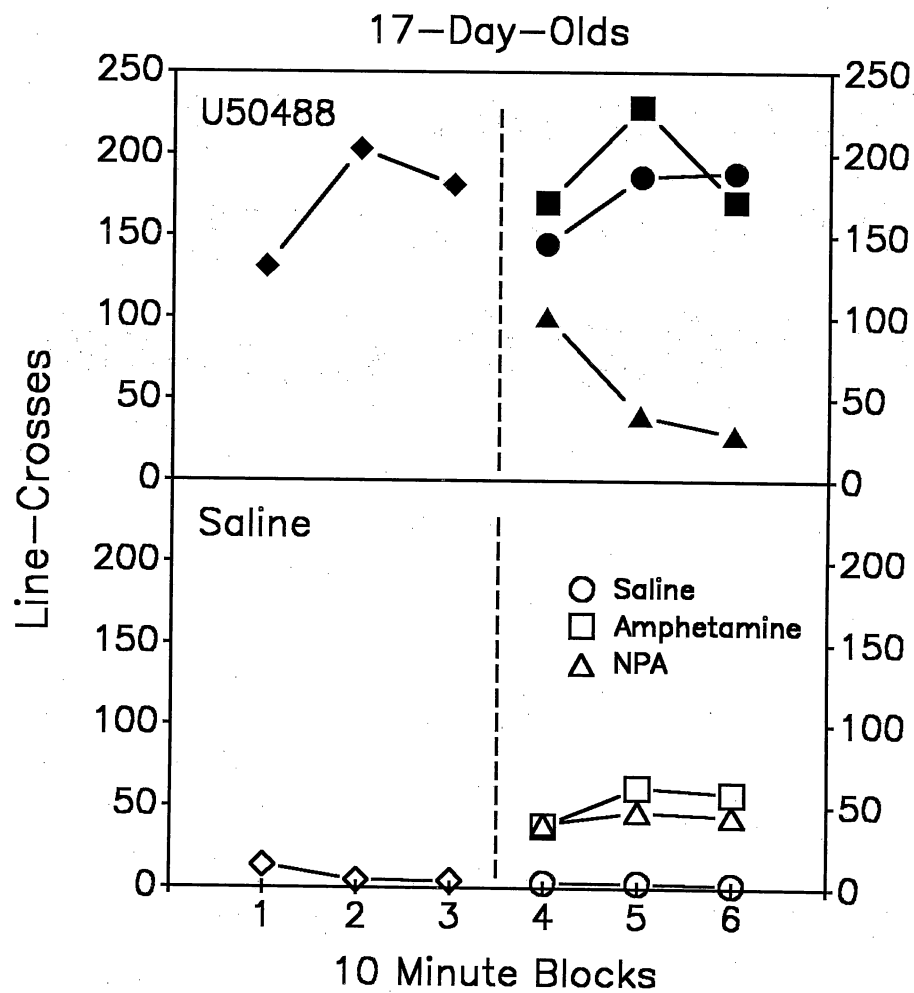


Table 6. Mean stereotyped sniffing counts (\pm SEM) of 17-day-old rats given U-50,488 (5.0 mg/kg) or saline immediately prior to behavioral testing. NPA (1.0 mg/kg), amphetamine (2.5 mg/kg) or saline were then administered 30 min into the testing session, with sniffing being assessed for an additional 30 min.

Drug Treatment	Stereotyped Sniffing Counts	
	First 30 Min	Second 30 Min
U-50,488/NPA	2.00 \pm 0.82	30.72 \pm 5.88 ^{AB}
Saline/NPA	0.75 \pm 0.49	42.62 \pm 5.28 ^B
U-50,488/Saline	1.75 \pm 0.52	1.25 \pm 0.86 ^A
Saline/Saline	1.12 \pm 0.66	0.62 \pm 0.49
U-50,488/Amphetamine	1.12 \pm 0.39	2.00 \pm 1.10 ^A
Saline/Amphetamine	1.50 \pm 0.53	8.37 \pm 2.34

^ASignificant pre main effect indicating that the U-50,488-pretreated rats were significantly different from the saline-pretreated rats, $P < 0.05$.

^BSignificant post main effect indicating that the NPA-treated rats were significantly different from the amphetamine- and saline-treated rats, $P < 0.05$.

Discussion

Results showed that amphetamine had only trivial effects on U50-488's stereotyped sniffing and locomotor activating effects. Unexpectedly, NPA dramatically *reduced* U-50,488-induced locomotor activity. When given alone, NPA increased the stereotyped sniffing of both U-50,488- and saline-pretreated rats.

Experiment 3b

The Effects of Amphetamine or NPA on U-50,488-Induced Behaviors in the 80-Day-Old Rat

Method

Subjects. Forty-eight male and female 80-day-old Sprague-Dawley albino rats (Harlan) were used.

Procedures. Six groups of 80-day-old rats ($n = 8$) received an acute injection of U-50,488 immediately prior to a 1 hr behavioral testing session. Subjects then received an acute injection of amphetamine, NPA, or saline 30 min into the behavioral testing session. One hour after behavioral testing, rats were anesthetized with phenobarbital and rapidly perfused with 4% paraformaldehyde testing for later analysis of Fos immunoreactivity.

Results

Line-crosses. During the initial three testing blocks there were no significant differences between 80-day-old rats treated with either U-50,488 or saline (see left panel, Figure 8). During the final three testing blocks amphetamine-treated rats had significantly more line-crosses than rats receiving saline (see right panel, Figure 8) [post main effect, $F(2,42)=9.40$, $P<0.001$; and Tukey tests, $P<0.05$]. During the fourth testing block rats given U-50,488 alone had significantly fewer line-crosses than all other rats

(see right panel, Figure 8) [Pre X Post X Time interaction, $F(4,84)=3.16$, $P<0.05$; and Tukey tests, $P<0.05$]. During the final two testing blocks, rats receiving amphetamine alone had significantly more line-crosses than the saline groups [Tukey tests, $P<0.05$].

Stereotyped sniffing. During the initial testing period there were no significant differences in sniffing between the U-50,488- and saline-treated rats (see Table 7). During the final testing period, rats pretreated with U-50,488 had significantly fewer stereotyped sniffing counts than saline-pretreated rats [pre main effect, $F(1,47)=5.68$, $P<0.05$]. This effect was entirely due to NPA's actions, as rats given NPA had significantly more stereotyped sniffing counts than their saline controls during the final testing period [post main effect, $F(2,47)=157.81$, $P<0.001$; and Tukey tests, $P<0.05$].

FIGURE CAPTION

Figure 8. Mean number of line-crosses during the 60-min behavioral testing session. The 80-day-old rats ($n = 8$) were injected with U-50,488 (5.0 mg/kg, s.c.) or saline immediately prior to behavioral testing. Rats were then injected with amphetamine (2.5 mg/kg, i.p.), NPA (1.0 mg/kg, i.p.) or saline 30 min into the testing session.

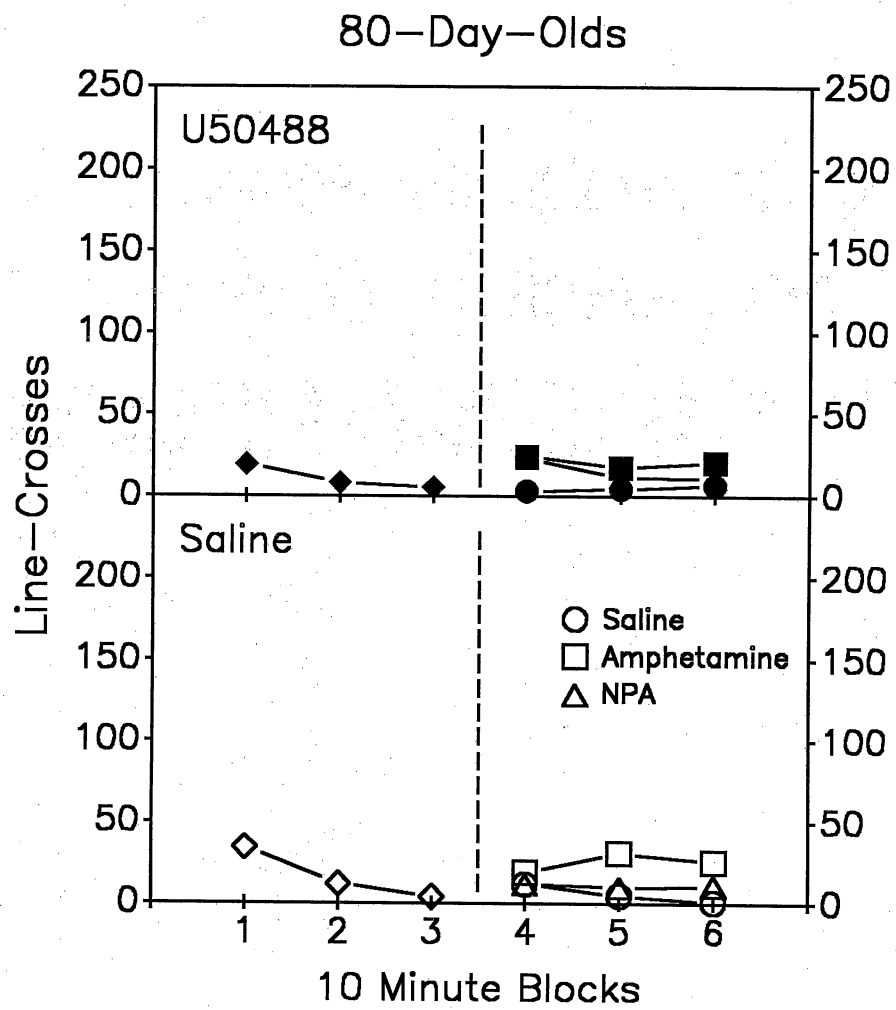


Table 7. Mean stereotyped sniffing counts (\pm SEM) of 80-day-old rats given U-50,488 (5.0 mg/kg) or saline immediately prior to behavioral testing. NPA (1.0 mg/kg), amphetamine (2.5 mg/kg) or saline were then administered 30 min into the testing session, with sniffing being assessed for an additional 30 min.

Drug Treatment	Stereotyped Sniffing Counts	
	First 30 Min	Second 30 Min
U-50,488/NPA	4.62 \pm 2.56	50.00 \pm 6.79 ^{AB}
Saline/NPA	5.38 \pm 1.39	66.13 \pm 2.83 ^B
U-50,488/Saline	3.88 \pm 0.90	3.38 \pm 1.84 ^A
Saline/Saline	6.38 \pm 2.04	5.38 \pm 2.31
U-50,488/Amphetamine	6.87 \pm 2.21	4.00 \pm 0.92 ^A
Saline/Amphetamine	7.63 \pm 2.23	6.12 \pm 2.91

^ASignificant pre main effect indicating that the U-50,488 groups were significantly different from the saline-pretreated groups, $P < 0.05$.

^BSignificant post main effect indicating that the NPA-treated groups were significantly different from all other groups, $P < 0.05$.

Discussion

As predicted, U-50,488 did not enhance the behavioral responding of the 80-day-old rat. Amphetamine did enhance the locomotor activity of the adult rats, while having no effect on stereotyped sniffing. Conversely, NPA increased the sniffing of 80-day-old rats, while having no effect on locomotor activity.

Experiment 3c

The Effects of U-50,488, Amphetamine, and NPA,
on Fos Immunoreactivity in the Preweanling and Adult Rat
Method

Subjects. Ninety-six male and female 17- and 80-day-old Sprague-Dawley rats (Harlan) were used. These rats were subjects from Experiments 3a and 3b.

Supplies. The primary antibody was a monoclonal antibody made to the N-terminal end of the Fos molecule in mouse myeloma cells (generously supplied from the laboratories of Frank Sharp and Stephen Sagar). This primary antibody recognizes Fos and not Fos-related antigens. The secondary antibody was a biotinylated mouse-anti-rat secondary antibody (Vector Laboratories, Inc., Burlingame, CA, USA). An avidin-biotin-horseradish peroxidase conjugate from an ABC horse kit was also used (Vector Laboratories, Inc.).

Procedures. Following a postfixation period, 100 micron sections were cut from each brain using a Vibratome 1000 (Ted Pella, Inc., Redding, CA, USA). The sections were washed three times with 0.1 M phosphate buffer (PB) before being incubated with the Fos primary antibody at a 1:50,000 dilution in HSS (0.1 M PB containing 2% horse serum, 0.1% triton-X100, and 0.1% bovine serum albumin). Sections were incubated in the primary antibody for 48-72 hr. Control sections from

each rat were run in the absence of the primary antibody. All sections were then washed three times in PB and incubated with a biotinylated mouse-anti-rat secondary antibody for 2 hr (1:10,000 dilution in HSS). Sections were washed three times in PB and incubated for 2 hr in avidin-biotin-horseradish peroxidase conjugate from ABC horse kits. Sections were then washed three more times and the Fos protein was visualized using 3,3'-diaminobenzidine tetrahydrochloride and hydrogen peroxide. Sections were then rinsed in PB and mounted on chrom-alum slides. The slides were then air dried, dehydrated, and coverslipped with permount. All washes lasted 5 min. This general procedure was based on the method of Sharp et al. (1989).

A total of 6 rats from each drug treatment group were randomly chosen for analysis of Fos immunoreactivity. Similar coronal sections from each rat were selected for quantitative analysis. The number of distinguishable immunoreactive nuclei present within the core and shell of the nucleus accumbens (1.7 A), the dorsal and ventral striatum (1.7 A), amygdala (3.8 P), septal area (0.3 P), olfactory tubercles (1.7 A), anterior cingulate cortex (1.7 A), stria medullaris (3.8 P), habenula (3.8 P), posterior hypothalamus (3.8 P), preoptic area (1.7 A), rhomboid nucleus (3.8 P), zona incerta (3.8 P), and piriform cortex (1.7 A) were

manually counted using a magnification of 40x. (The numbers in parentheses represent sections either anterior or posterior to bregma, according to the rat brain atlas of Pellegrino & Cushman, 1967.) At each region, specific sample areas were counted with the size of the sample area being 10 X 10 mm.

Results

Effects of U-50,488, amphetamine, and NPA on Fos immunoreactivity in 17-day-old rats. U-50,488 enhanced Fos immunoreactivity in specific brain regions of the preweanling rat (see Figures 9-21). That is, compared to rats given saline, U-50,488 significantly enhanced the number of Fos-positive nuclei in the striatum, olfactory tubercles, piriform cortex, habenula, preoptic area, amygdala, stria medullaris, cingulate cortex, and septal area [$t(10)=3.73$, $P<0.01$; $t(10)=3.21$, $P<0.01$; $t(10)=2.24$, $P<0.05$; $t(10)=3.09$, $P<0.05$; $t(10)=3.72$, $P<0.01$; $t(10)=2.99$, $P<0.05$; $t(10)=4.28$, $P<0.01$; $t(10)=3.52$, $P<0.01$; $t(10)=3.44$, $P<0.01$, respectively]. Moreover, collapsed across the posttreatment, 17-day-old rats given U-50,488 had significantly more Fos-positive nuclei than saline-treated rats in the striatum, piriform cortex, habenula, preoptic area, amygdala, and septal area [pre main effects, $F(1,35)=17.29$, $P<0.001$; $F(1,35)=7.33$, $P<0.05$; $F(1,35)=34.65$, $P<0.001$; $F(1,35)=26.77$, $P<0.001$; $F(1,35)=5.74$, $P<0.05$;

$F(1,35)=23.42$, $P<0.001$, respectively].

Amphetamine and NPA also had their own effects on Fos immunoreactivity. For example, amphetamine significantly attenuated U-50,488-induced Fos expression in the olfactory tubercles (see Figure 10) [Pre X Post interaction, $F(2,35)=5.38$, $P<0.01$; and Tukey tests, $P<0.05$]. Similarly, NPA significantly depressed U-50,488-induced Fos expression in the striatum [Tukey tests, $P<0.05$]. Conversely, amphetamine and NPA enhanced the number of Fos-positive nuclei in the piriform cortex (see Figure 13) [post main effect, $F(1,35)=3.17$, $P<0.05$]. In the cingulate cortex, 17-day-old rats given amphetamine alone had significantly more Fos-positive nuclei than rats in the saline control group [Pre X Post interaction, $F(2,35)=3.58$, $P<0.05$; and Tukey tests, $P<0.05$].

Effects of amphetamine, NPA, and U-50,488 on Fos immunoreactivity in 80-day-old rats. In general, U-50,488 did not affect Fos expression in 80-day-old rats. The one exception was the habenula, where there were more Fos-positive nuclei in the U-50,488-pretreated rats than in the saline-pretreated rats (see Figure 22) [pre main effect, $F(1,35)=6.62$, $P<0.05$].

NPA significantly increased Fos expression in the piriform cortex, an effect attenuated by U-50,488 pretreatment (see Figure 23) [Pre X Post interaction,

$F(2,35)=6.37$, $P<0.01$; and Tukey tests, $P<0.05$]. NPA also reduced the mean number of Fos-positive nuclei in the olfactory tubercles, however this was apparent in both the saline- and U-50,488-pretreated rats (see Figure 24) [post main effect, $F(2,35)=4.34$, $P<0.05$; and Tukey tests, $P<0.05$].

Amphetamine only affected Fos expression in the zona incerta. In this particular region, amphetamine decreased the number of Fos-positive nuclei relative to the saline-treated rats (see Figure 25) [post main effect, $F(2,35)=3.87$, $P<0.05$; and Tukey tests, $P<0.05$].

FIGURE CAPTION

Figure 9. Mean number (+SEM) of Fos-positive nuclei throughout the striatum of the 17-day-old rat ($n = 6$). The rats were sacrificed 60 min after behavioral testing and were the same rats as those described in Figure 7.

^aSignificant t -test indicating that the U-50,488/saline group was significantly different from the saline/saline group, $P < 0.01$. ^ASignificant pretreatment main effect indicating that U-50,488-pretreated rats were significantly different from saline-pretreated rats, $P < 0.001$.

17-Day-Olds

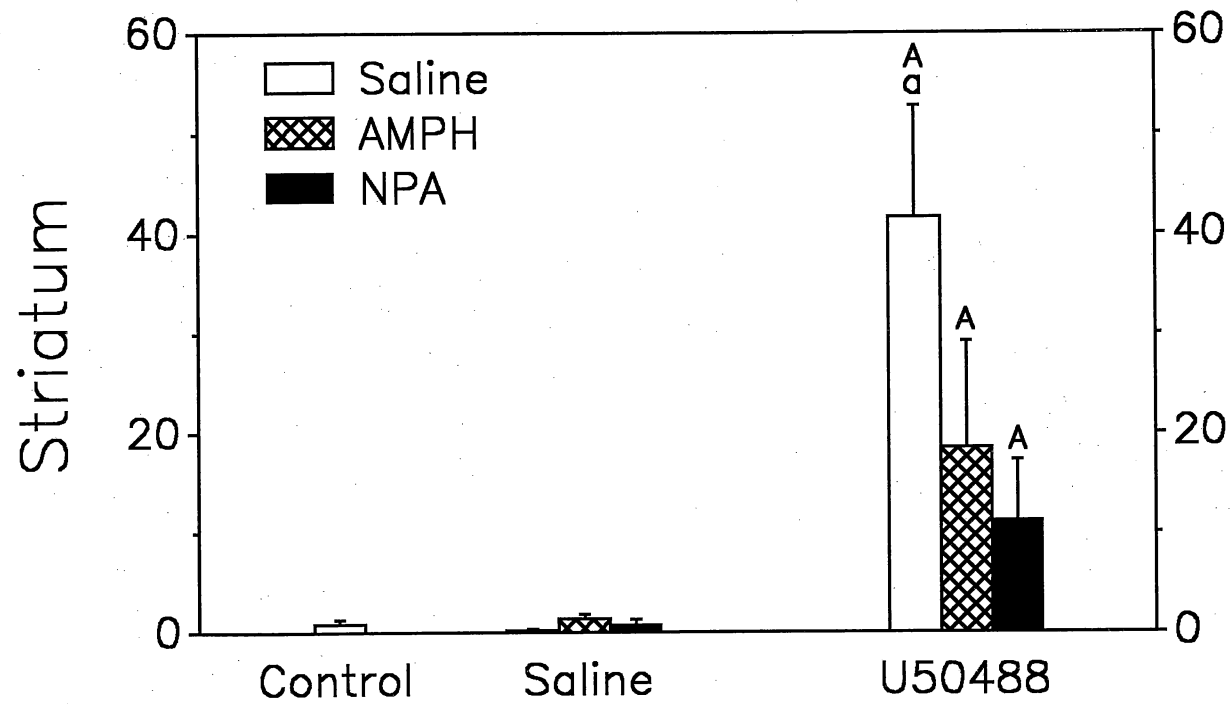


FIGURE CAPTION

Figure 10. Mean number (+SEM) of Fos-positive nuclei throughout the olfactory tubercles of the 17-day-old rat ($n = 6$). The rats were sacrificed 60 min after behavioral testing and were the same rats as those described in Figure 7. ^aSignificant Pre X Post interaction indicating that the U-50,488/saline and U-50,488/NPA groups were significantly different from their respective saline-pretreated control groups, $P < 0.05$. ^bSignificant Pre X Post interaction indicating that the U-50,488/amphetamine group was significantly different from the U-50,488/saline group, $P < 0.05$.

17-Day-Olds

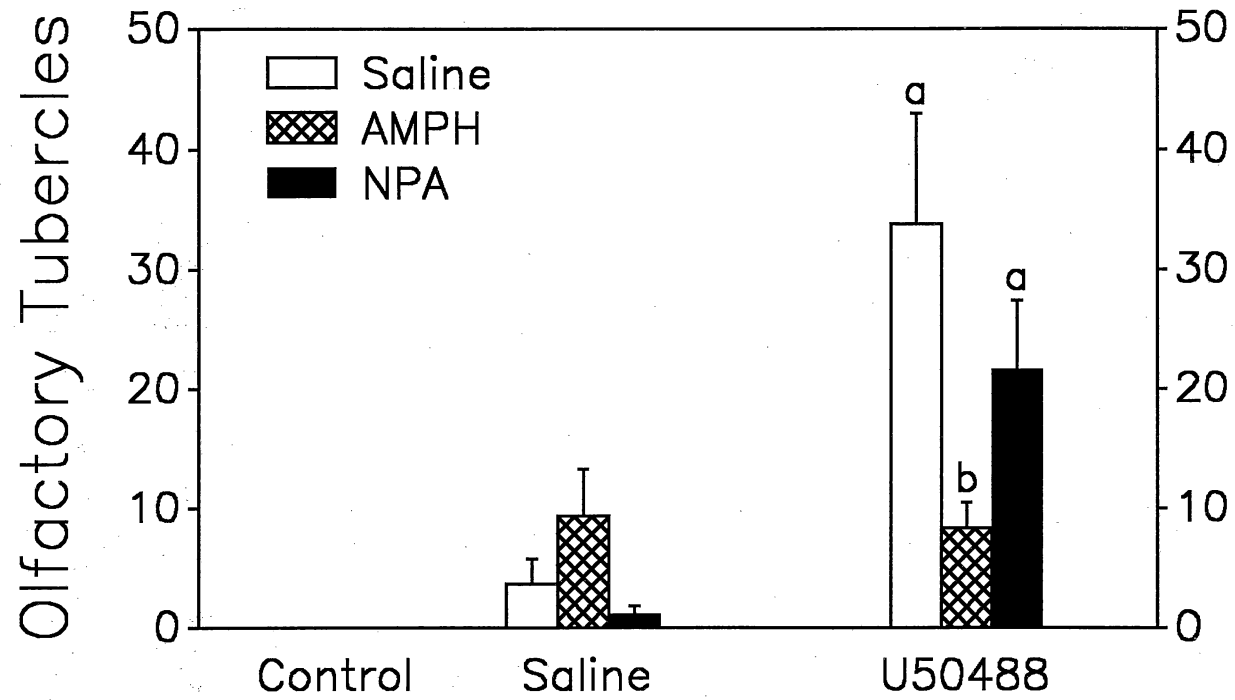


FIGURE CAPTION

Figure 11. Mean number (+SEM) of Fos-positive nuclei throughout the piriform cortex of the 17-day-old rat ($n = 6$). The rats were sacrificed 60 min after behavioral testing and were the same rats as those described in Figure 7. ^aSignificant t -test indicating that the U-50,488/saline group was significantly different from the saline/saline group, $P < 0.05$. ^ASignificant pre main effect indicating that U-50,488-pretreated rats were significantly different from saline-pretreated rats, $P < 0.05$. ^BSignificant post main effect indicating that the amphetamine- and NPA-treated rats were significantly different from their saline-treated controls, $P < 0.05$.

17-Day-Olds

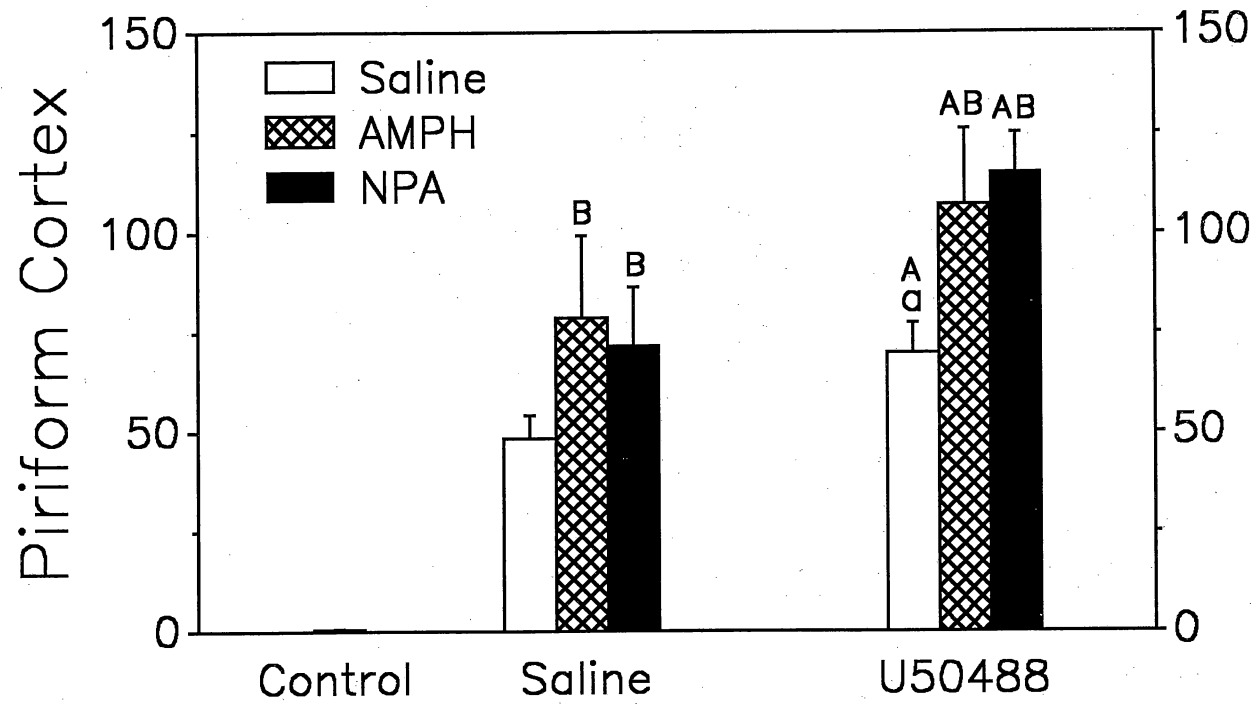


FIGURE CAPTION

Figure 12. Mean number (+SEM) of Fos-positive nuclei throughout the habenula of the 17-day-old rat ($n = 6$). The rats were sacrificed 60 min after behavioral testing and were the same rats as those described in Figure 7. ^aSignificant t -test indicating that the U-50,488/saline group was significantly different from the saline/saline group, $P < 0.05$. [^]Significant pre main effect indicating that U-50,488-pretreated rats were significantly different from the saline-pretreated rats, $P < 0.001$.

17-Day-Olds

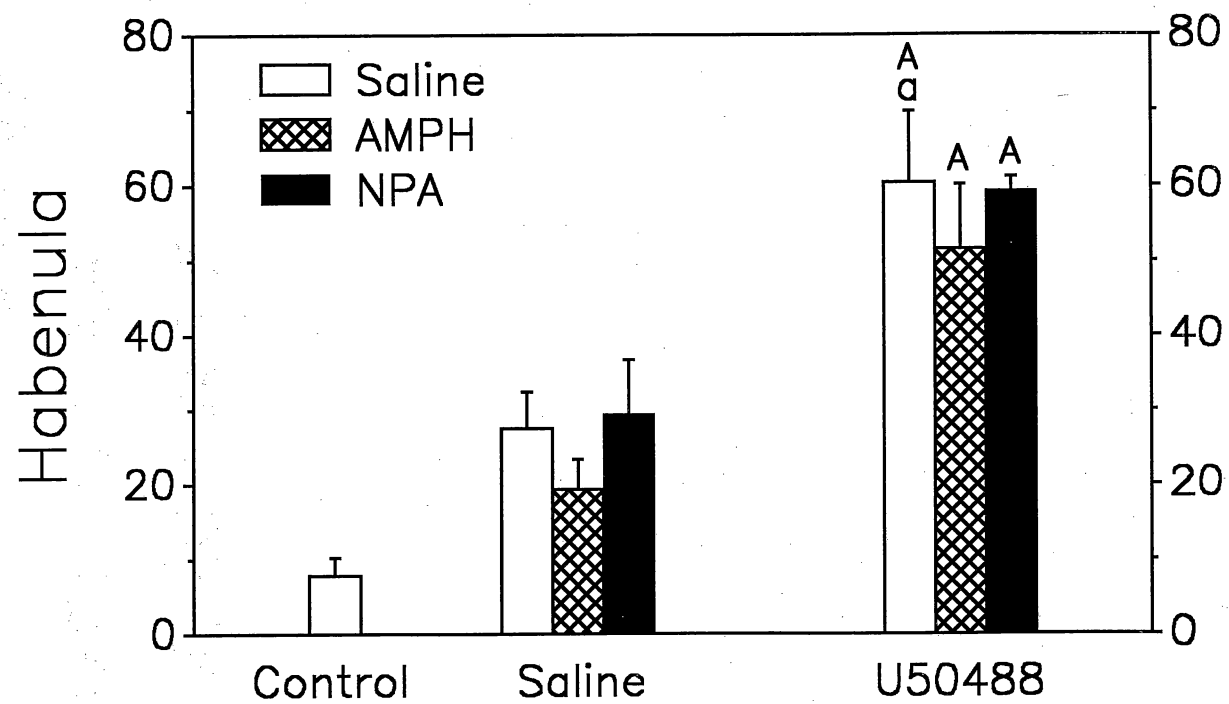


FIGURE CAPTION

Figure 13. Mean number (+SEM) of Fos-positive nuclei throughout the preoptic area of the 17-day-old rat ($n = 6$). The rats were sacrificed 60 min after behavioral testing and were the same rats as those described in Figure 7. ^aSignificant t -test indicating that the U-50,488/saline group was significantly different from the saline/saline group, $P < 0.01$. ^ASignificant pre main effect indicating that U-50,488-pretreated rats were significantly different from saline-pretreated rats, $P < 0.001$.

17-Day-Olds

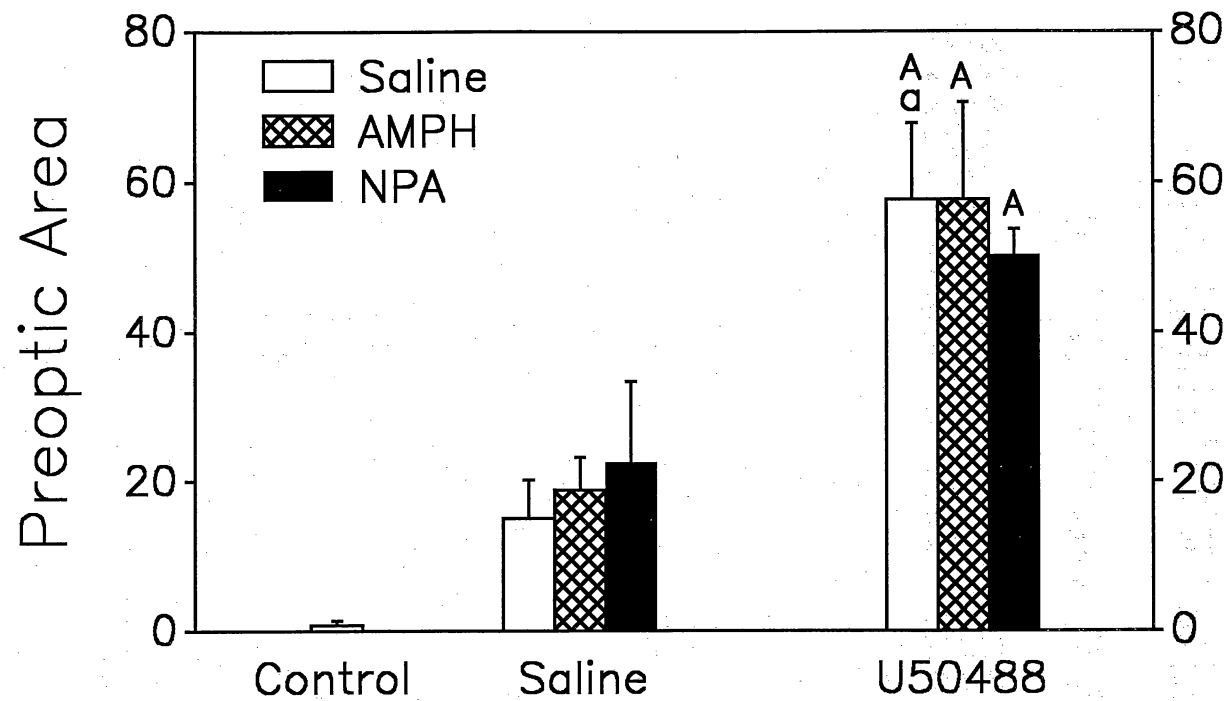


FIGURE CAPTION

Figure 14. Mean number (+SEM) of Fos-positive nuclei throughout the amygdala of the 17-day-old rat ($n = 6$). The rats were sacrificed 60 min after behavioral testing and were the same rats as those described in Figure 7.

^aSignificant t -test indicating that the U-50,488/saline group was significantly different from the saline/saline group, $P < 0.05$. ^ASignificant pre main effect indicating that U-50,488-pretreated rats were significantly different from saline-pretreated rats, $P < 0.05$.

17-Day-Olds

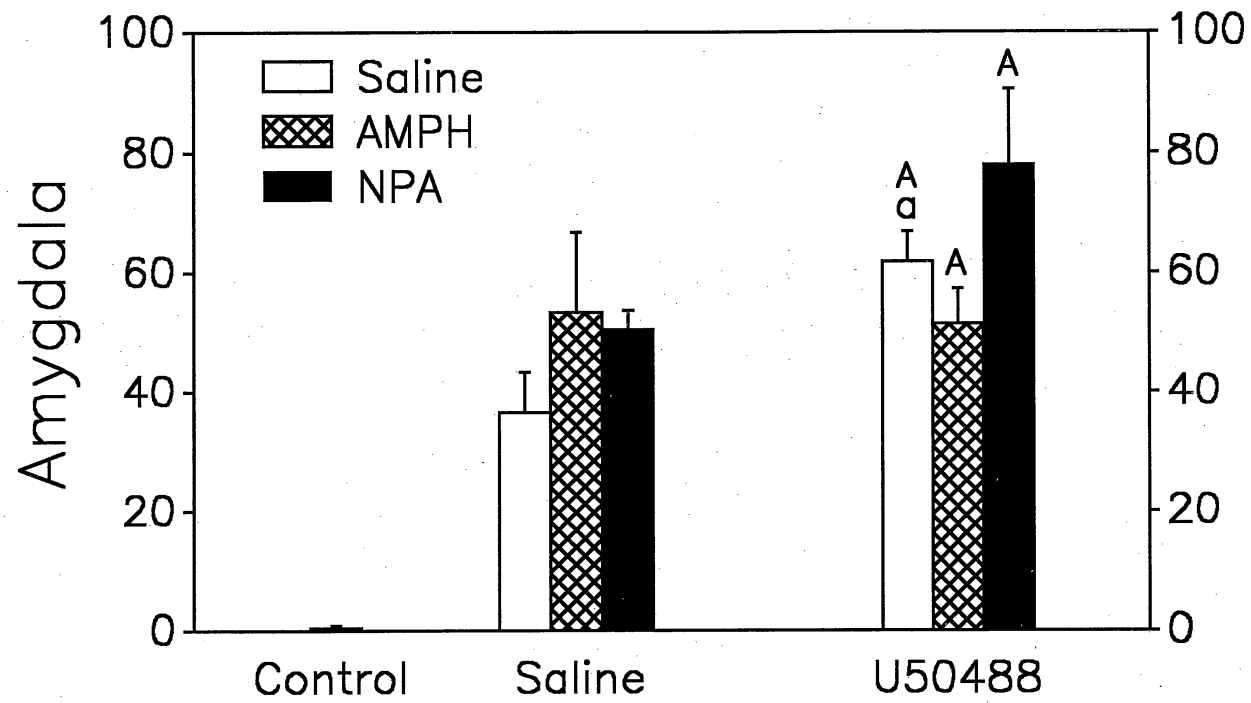


FIGURE CAPTION

Figure 15. Mean number (+SEM) of Fos-positive nuclei throughout the stria medullaris of the 17-day-old rat ($n = 6$). The rats were sacrificed 60 min after behavioral testing and were the same rats as those described in Figure 7. ^aSignificant t -test indicating that the U-50,488/saline group was significantly different from the saline/saline group, $P < 0.01$.

17-Day-Olds

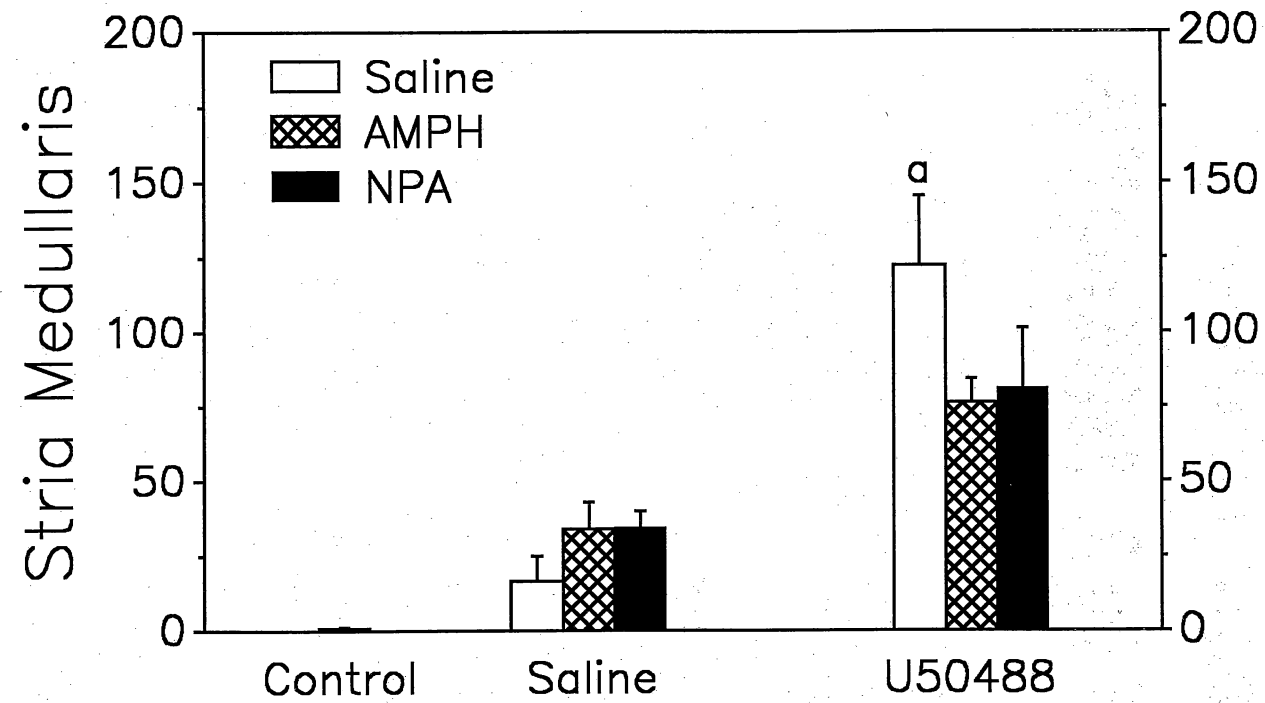


FIGURE CAPTION

Figure 16. Mean number (+SEM) of Fos-positive nuclei throughout the cingulate cortex of the 17-day-old rat ($n = 6$). The rats were sacrificed 60 min after behavioral testing and were the same rats as those described in Figure 7. ^aSignificant t -test indicating that the U-50,488/saline group was significantly different from the saline/saline group, $P < 0.05$. ^bSignificant Pre X Post interaction indicating that the saline/amphetamine group was significantly different from the saline/saline group, $P < 0.05$.

17-Day-Olds

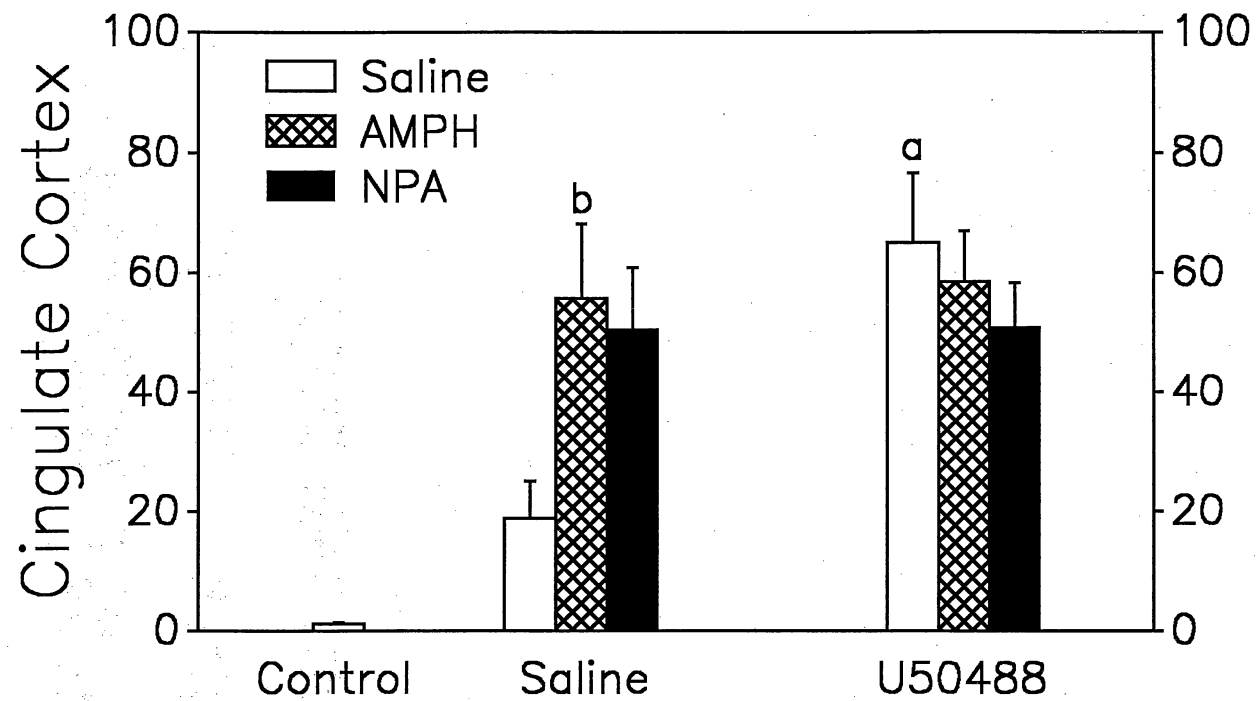


FIGURE CAPTION

Figure 17. Mean number (+SEM) of Fos-positive nuclei throughout the septal area of the 17-day-old rat ($n = 6$). The rats were sacrificed 60 min after behavioral testing and were the same rats as those described in Figure 7. ^aSignificant t -test indicating that the U-50,488/saline group was significantly different from the saline/saline group, $P < 0.01$. ^ASignificant pre main effect indicating that U-50,488-pretreated rats were significantly different from saline-pretreated rats, $P < 0.001$.

17-Day-Olds

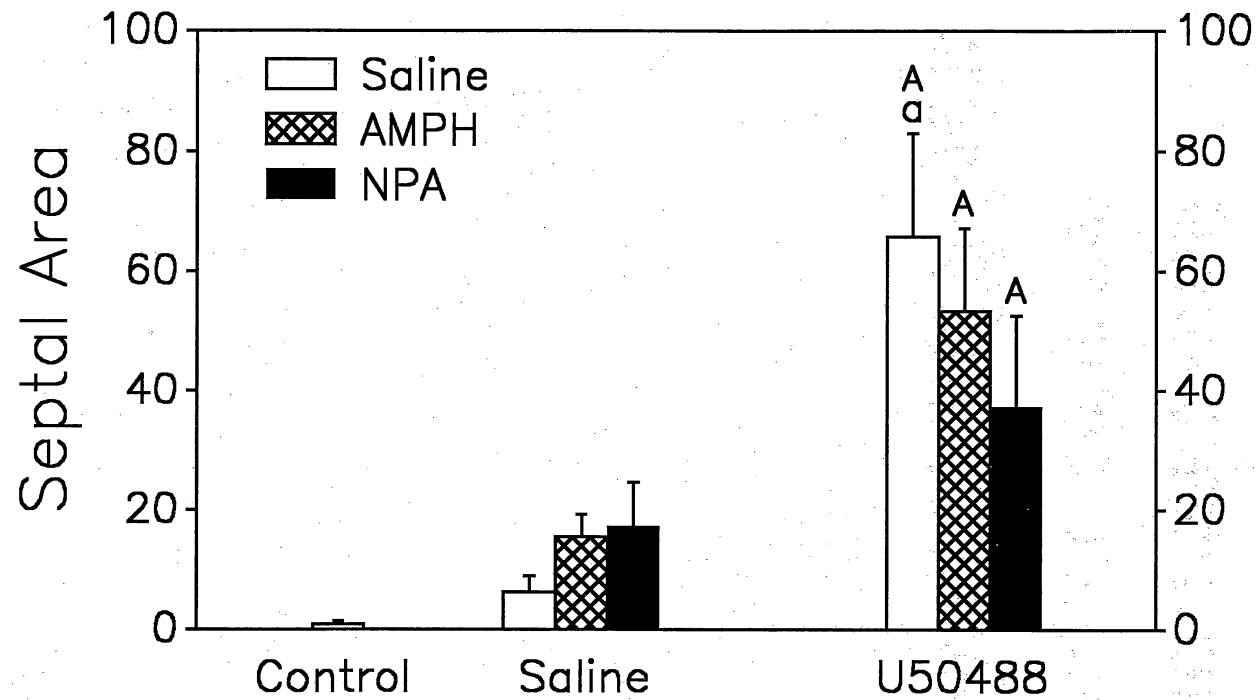


FIGURE CAPTION

Figure 18. Mean number (+SEM) of Fos-positive nuclei throughout the nucleus accumbens of the 17-day-old rat ($n = 6$). The rats were sacrificed 60 min after behavioral testing and were the same rats as those described in Figure 7.

17-Day-Olds

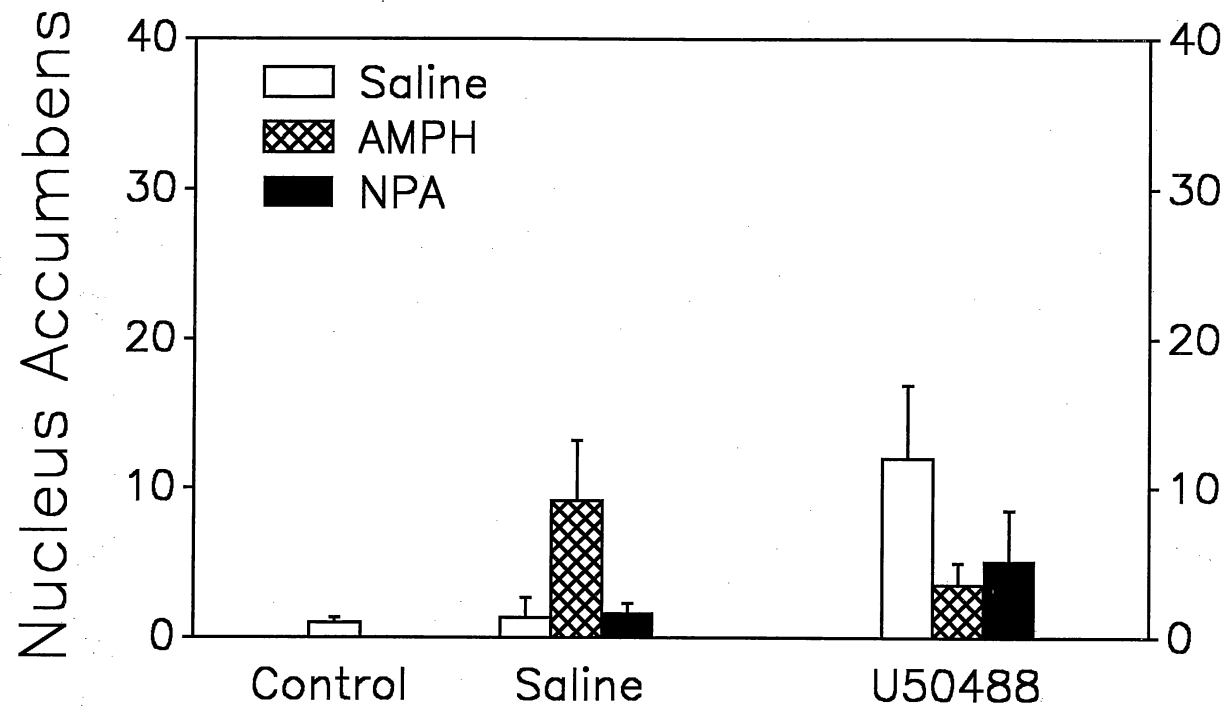


FIGURE CAPTION

Figure 19. Mean number (+SEM) of Fos-positive nuclei throughout the posterior hypothalamus of the 17-day-old rat ($n = 6$). The rats were sacrificed 60 min after behavioral testing and were the same rats as those described in Figure 7.

Posterior Hypothalamus

17-Day-Olds

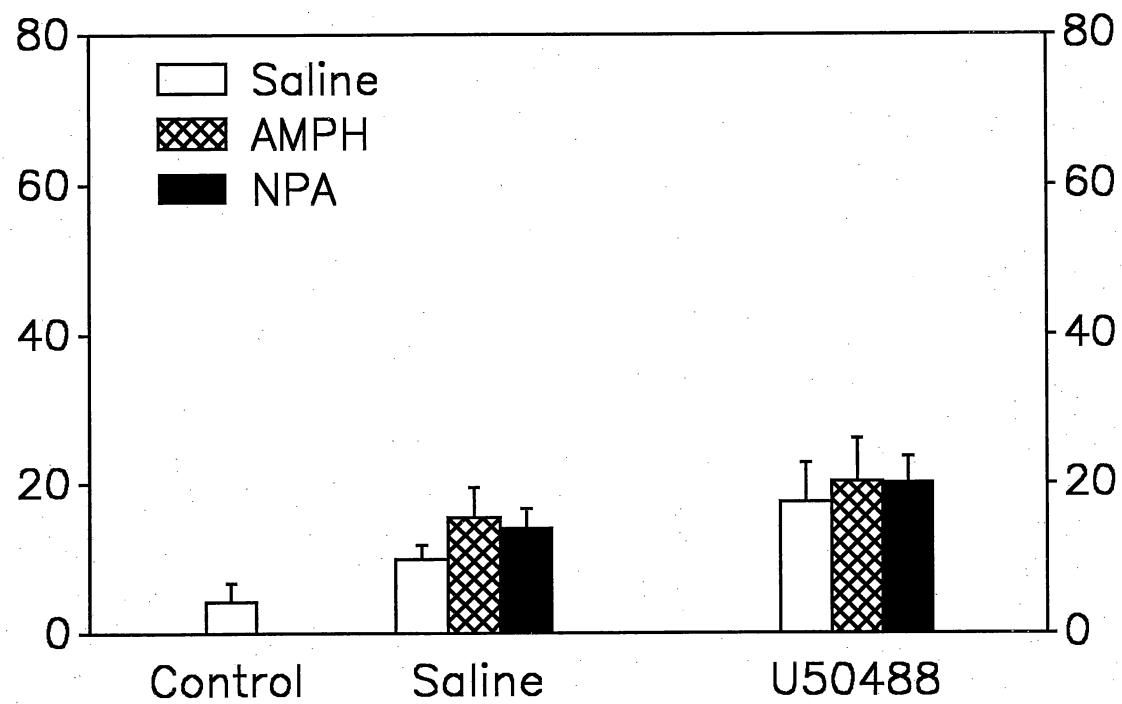


FIGURE CAPTION

Figure 20. Mean number (+SEM) of Fos-positive nuclei throughout the rhomboid nucleus of the 17-day-old rat ($n = 6$). The rats were sacrificed 60 min after behavioral testing and were the same rats as those described in Figure 7.

17-Day-Olds

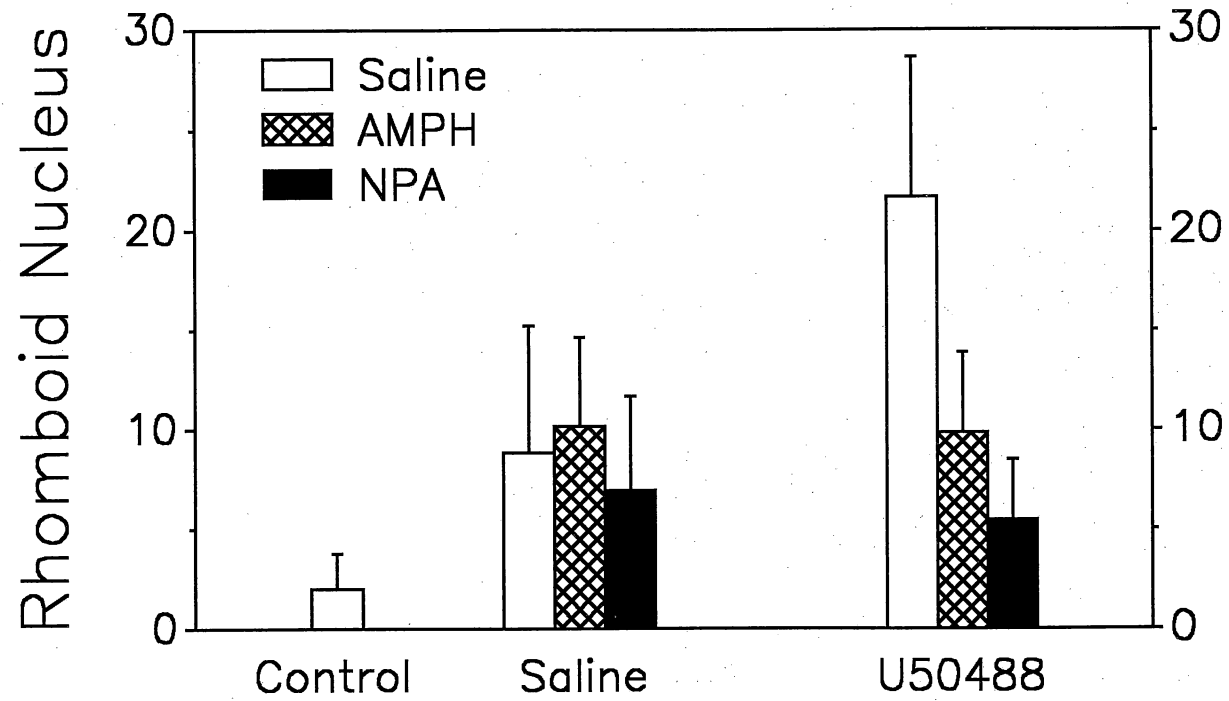


FIGURE CAPTION

Figure 21. Mean number (+SEM) of Fos-positive nuclei throughout the zona incerta of the 17-day-old rat ($n = 6$). The rats were sacrificed 60 min after behavioral testing and were the same rats as those described in Figure 7.

17-Day-Olds

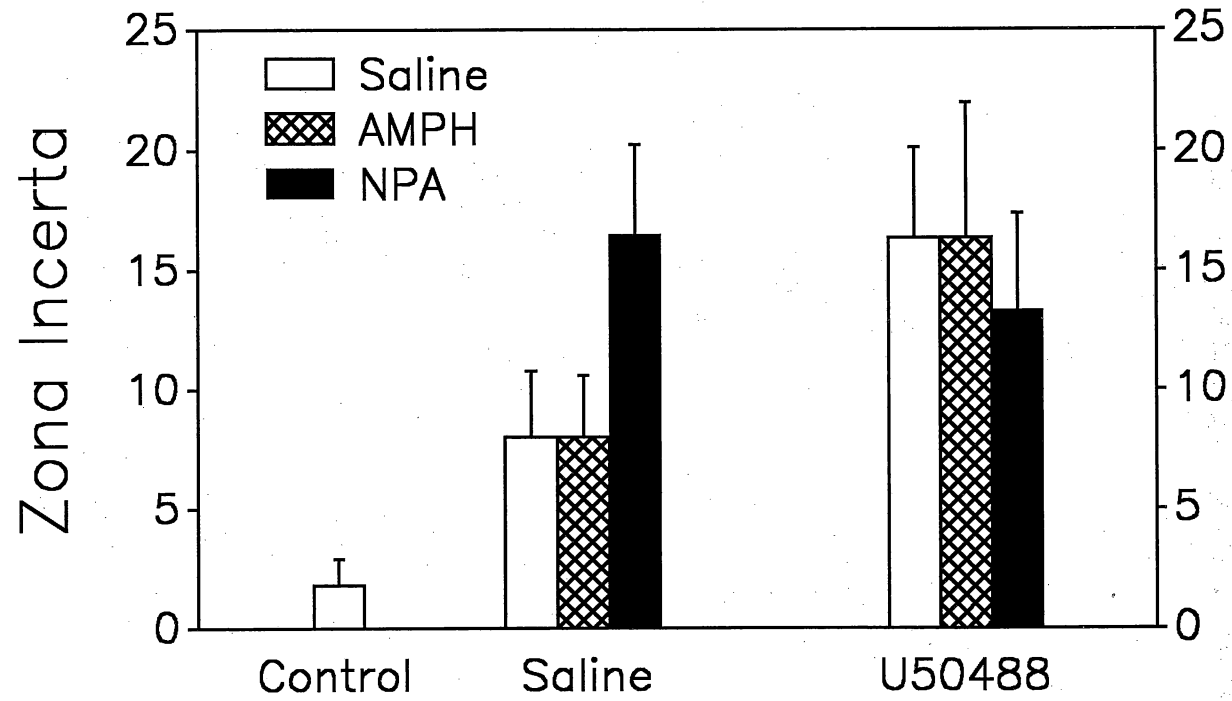


FIGURE CAPTION

Figure 22. Mean number (+SEM) of Fos-positive nuclei throughout the habenula of the 80-day-old rat ($n = 6$). The rats were sacrificed 60 min after behavioral testing and were the same rats as those described in Figure 8. ^ASignificant pre main effect indicating that U-50,488-pretreated rats were significantly different from saline-pretreated rats, $P < 0.001$.

80-Day-Olds

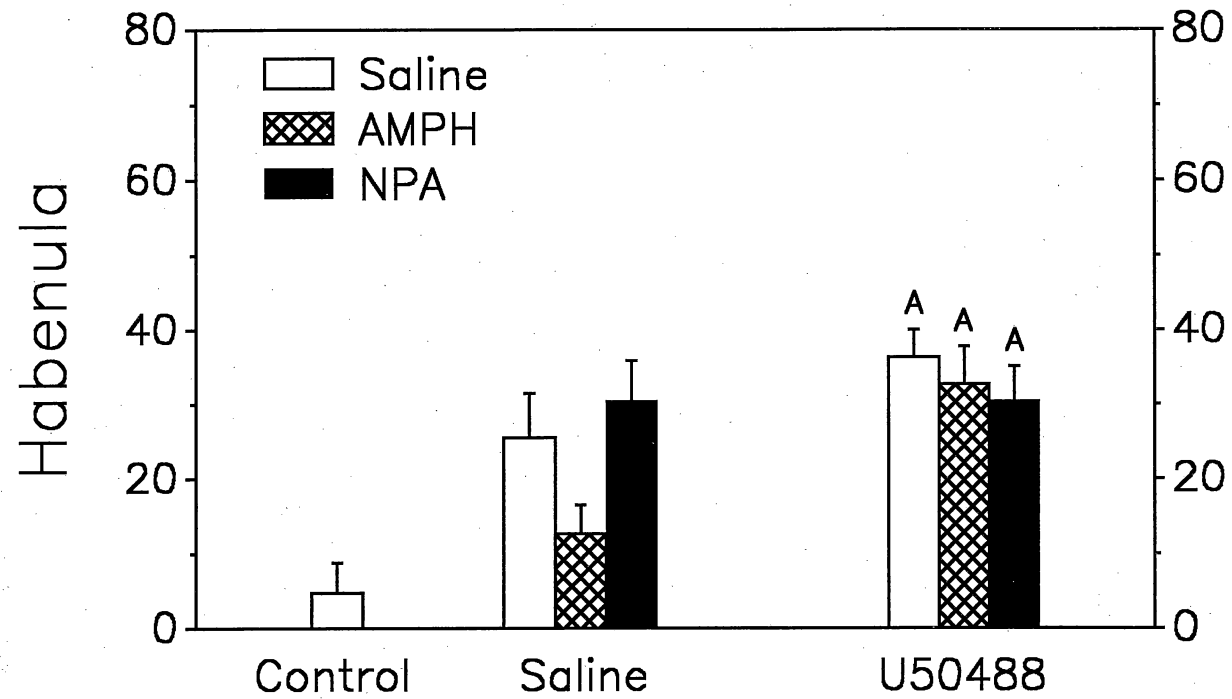


FIGURE CAPTION

Figure 23. Mean number (+SEM) of Fos-positive nuclei throughout the piriform cortex of the 80-day-old rat ($n = 6$). The rats were sacrificed 60 min after behavioral testing and were the same rats as those described in Figure 8. ^aSignificant Pre X Post interaction indicating that the U-50,488/NPA group was significantly different from the saline/NPA group, $P < 0.05$. ^bSignificant Pre X Post interaction indicating that the saline/NPA group was significantly different from the saline/saline group, $P < 0.05$.

80-Day-Olds

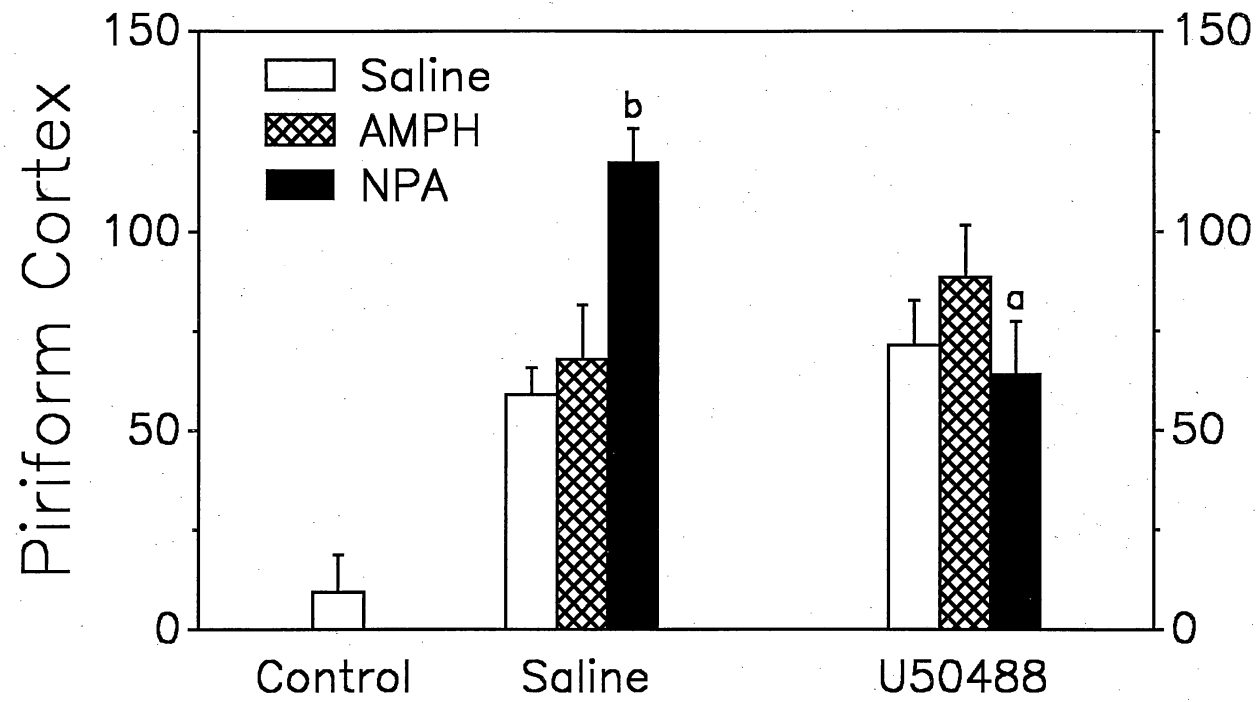


FIGURE CAPTION

Figure 24. Mean number (+SEM) of Fos-positive nuclei throughout the olfactory tubercles of the 80-day-old rat ($n = 6$). The rats were sacrificed 60 min after behavioral testing and were the same rats as those described in Figure 8. ^BSignificant post main effect indicating that the NPA-treated rats were significantly different from their saline-treated controls, $P < 0.05$.

80-Day-Olds

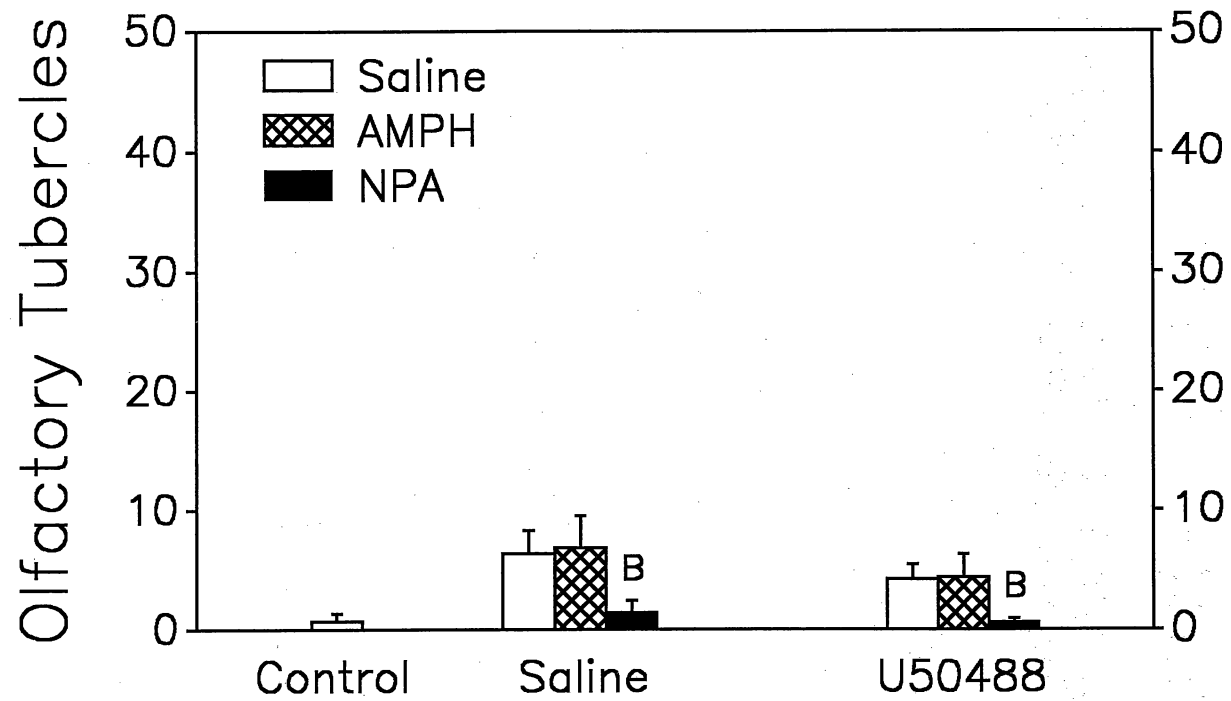


FIGURE CAPTION

Figure 25. Mean number (+SEM) of Fos-positive nuclei throughout the zona incerta of the 80-day-old rat ($n = 6$). The rats were sacrificed 60 min after behavioral testing and were the same rats as those described in Figure 8. ^BSignificant post main effect indicating that the NPA-treated rats were significantly different from their saline-treated controls, $P < 0.05$.

80-Day-Olds

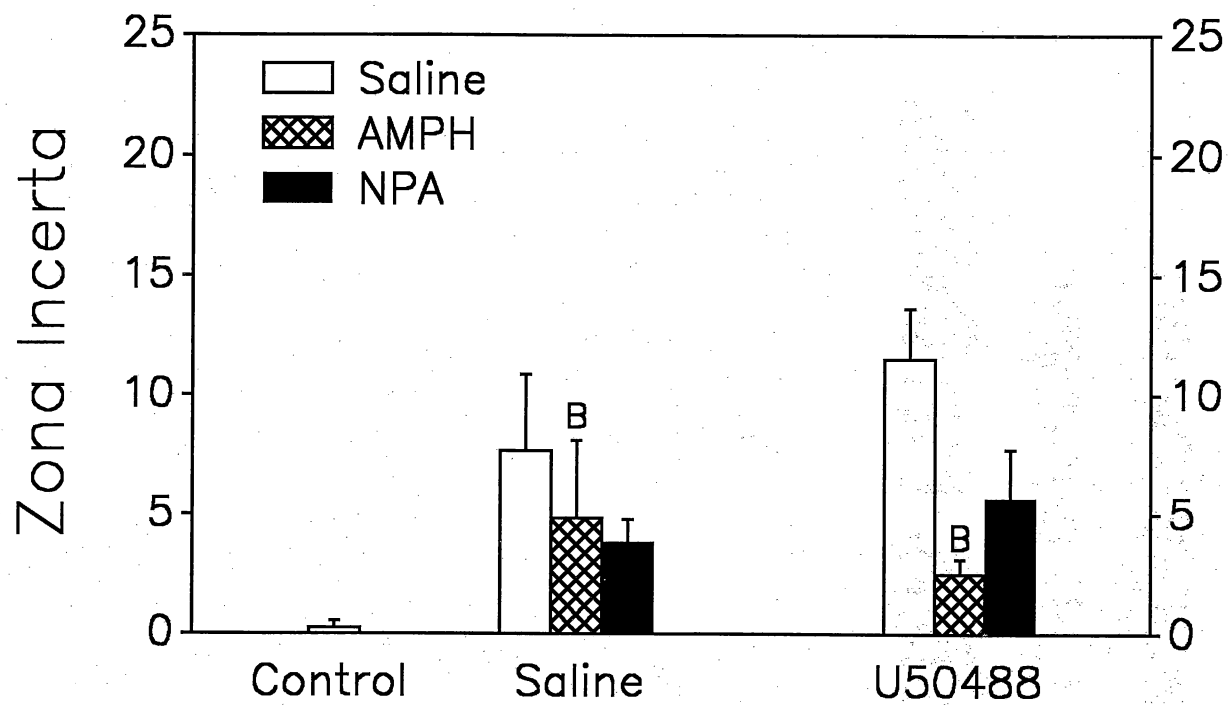


FIGURE CAPTION

Figure 26. Mean number (+SEM) of Fos-positive nuclei throughout the striatum of the 80-day-old rat ($n = 6$). The rats were sacrificed 60 min after behavioral testing and were the same rats as those described in Figure 8.

80-Day-Olds

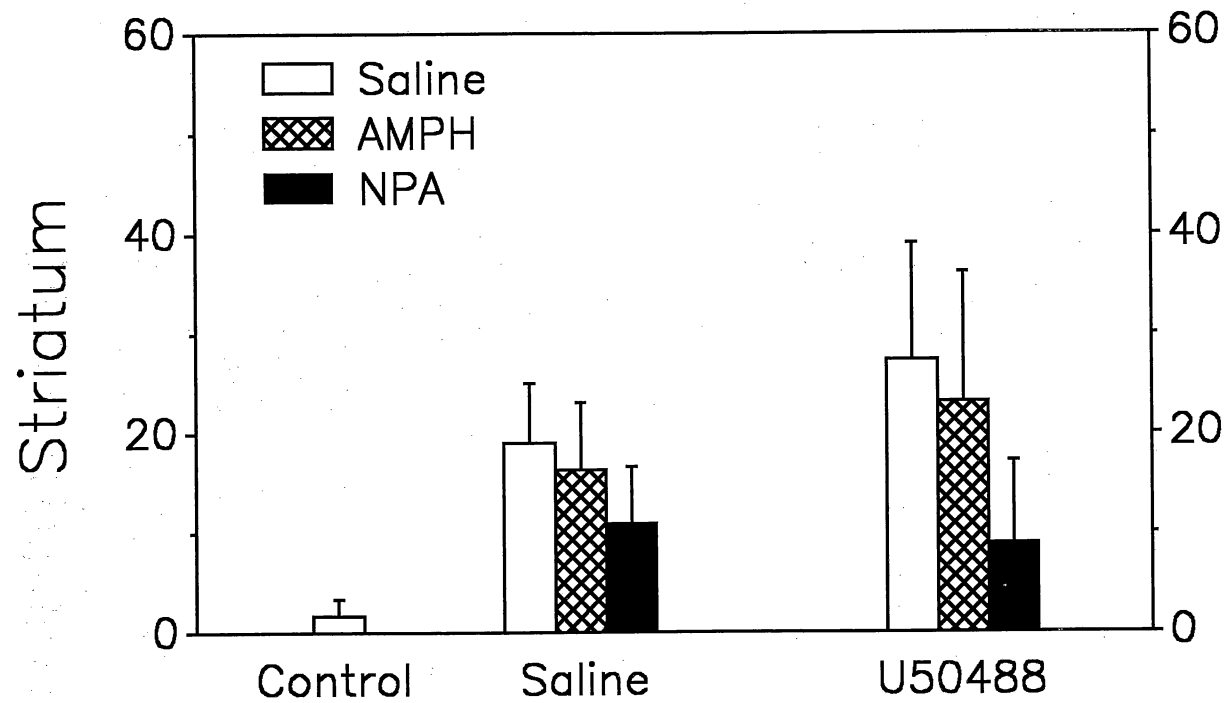


FIGURE CAPTION

Figure 27. Mean number (+SEM) of Fos-positive nuclei throughout the preoptic area of the 80-day-old rat ($n = 6$). The rats were sacrificed 60 min after behavioral testing and were the same rats as those described in Figure 8.

80-Day-Olds

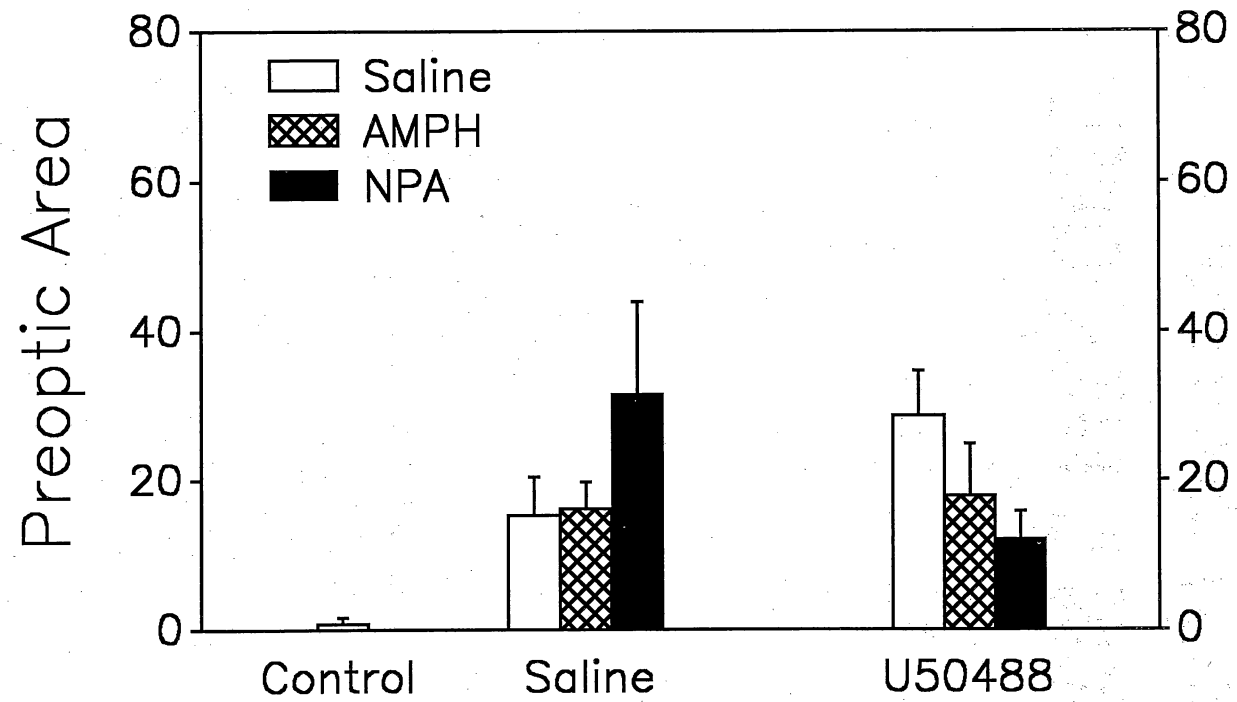


FIGURE CAPTION

Figure 28. Mean number (+SEM) of Fos-positive nuclei throughout the amygdala of the 80-day-old rat ($n = 6$). The rats were sacrificed 60 min after behavioral testing and were the same rats as those described in Figure 8.

80-Day-Olds

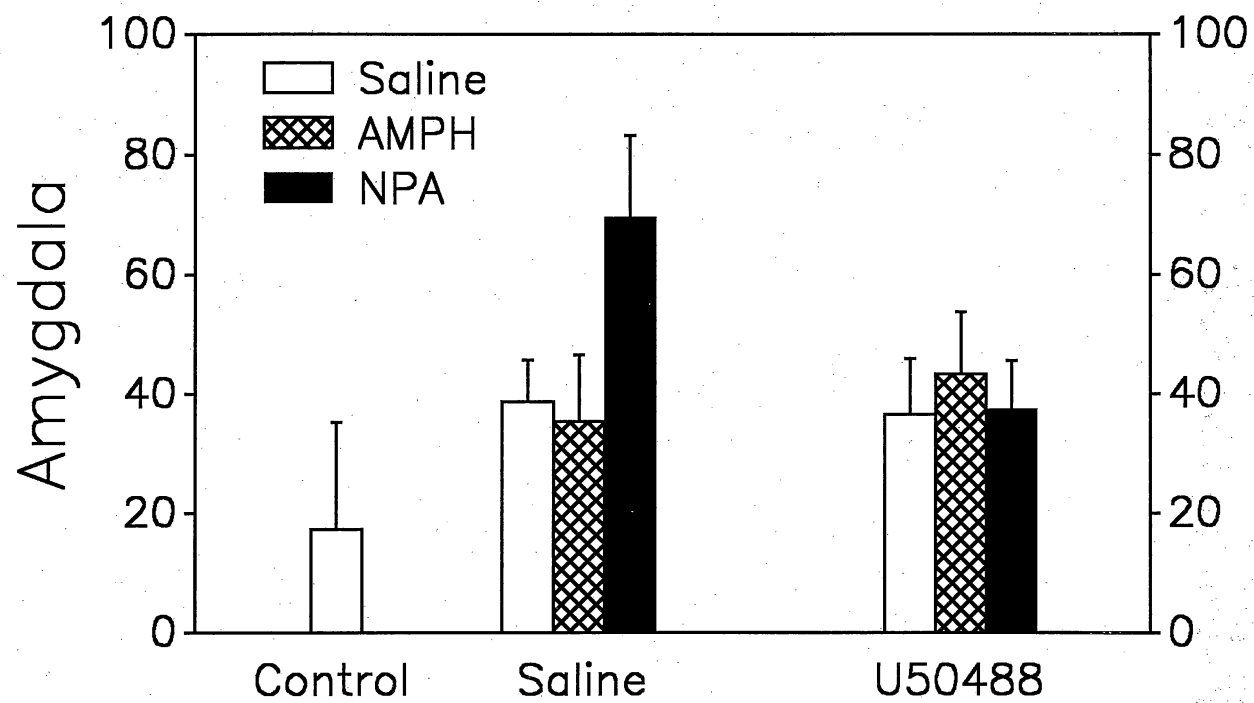


FIGURE CAPTION

Figure 29. Mean number (+SEM) of Fos-positive nuclei throughout the septal area of the 80-day-old rat ($n = 6$). The rats were sacrificed 60 min after behavioral testing and were the same rats as those described in Figure 8.

80-Day-Olds

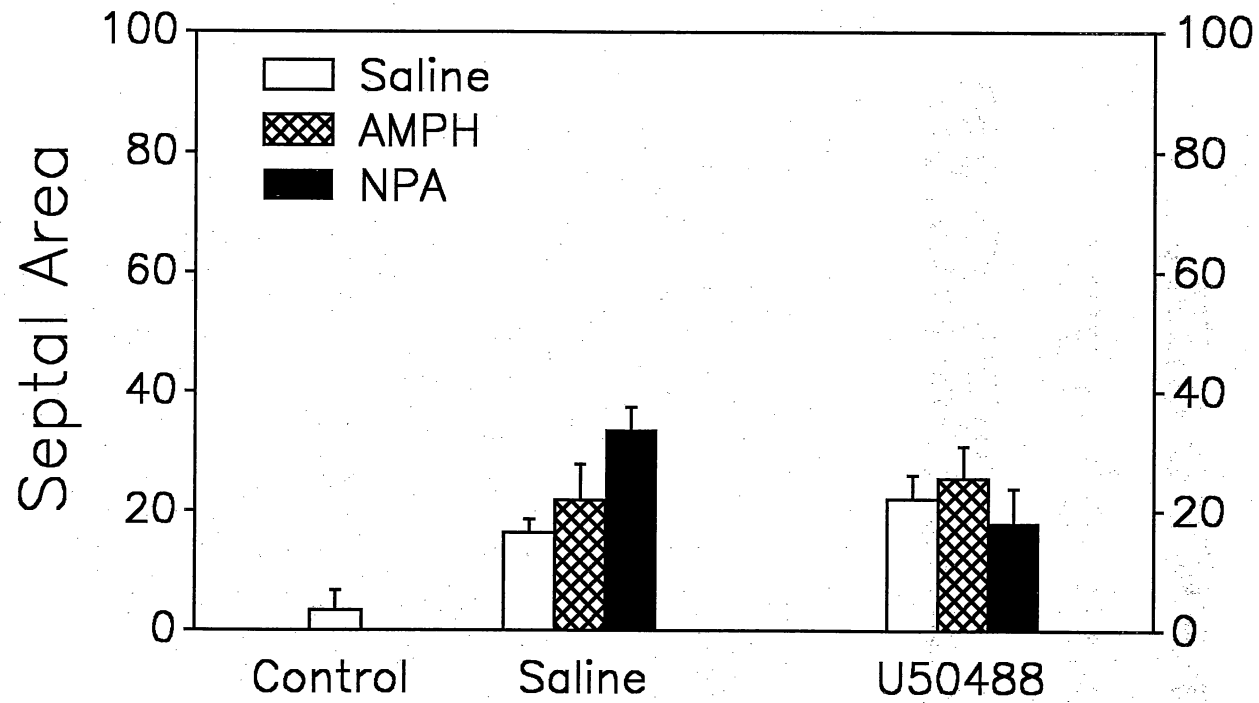


FIGURE CAPTION

Figure 30. Mean number (+SEM) of Fos-positive nuclei throughout the cingulate cortex of the 80-day-old rat ($n = 6$). The rats were sacrificed 60 min after behavioral testing and were the same rats as those described in Figure 8.

80-Day-Olds

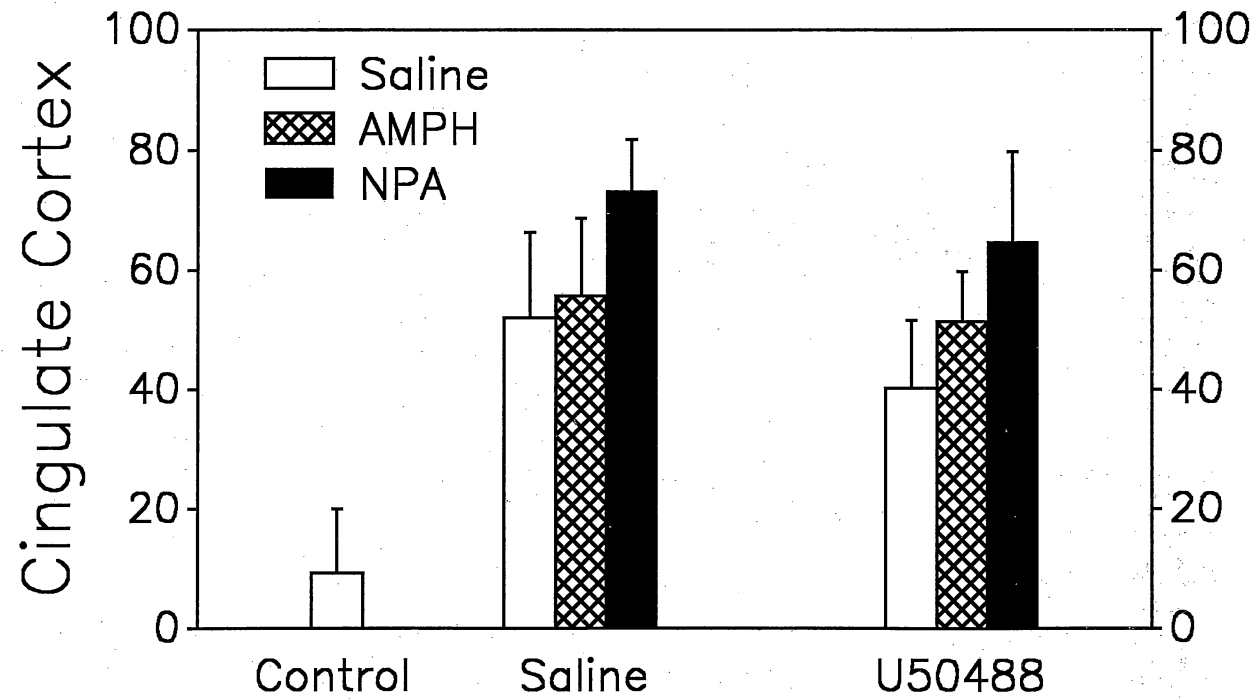


FIGURE CAPTION

Figure 31. Mean number (+SEM) of Fos-positive nuclei throughout the stria medullaris of the 80-day-old rat ($n = 6$). The rats were sacrificed 60 min after behavioral testing and were the same rats as those described in Figure 8.

80-Day-Olds

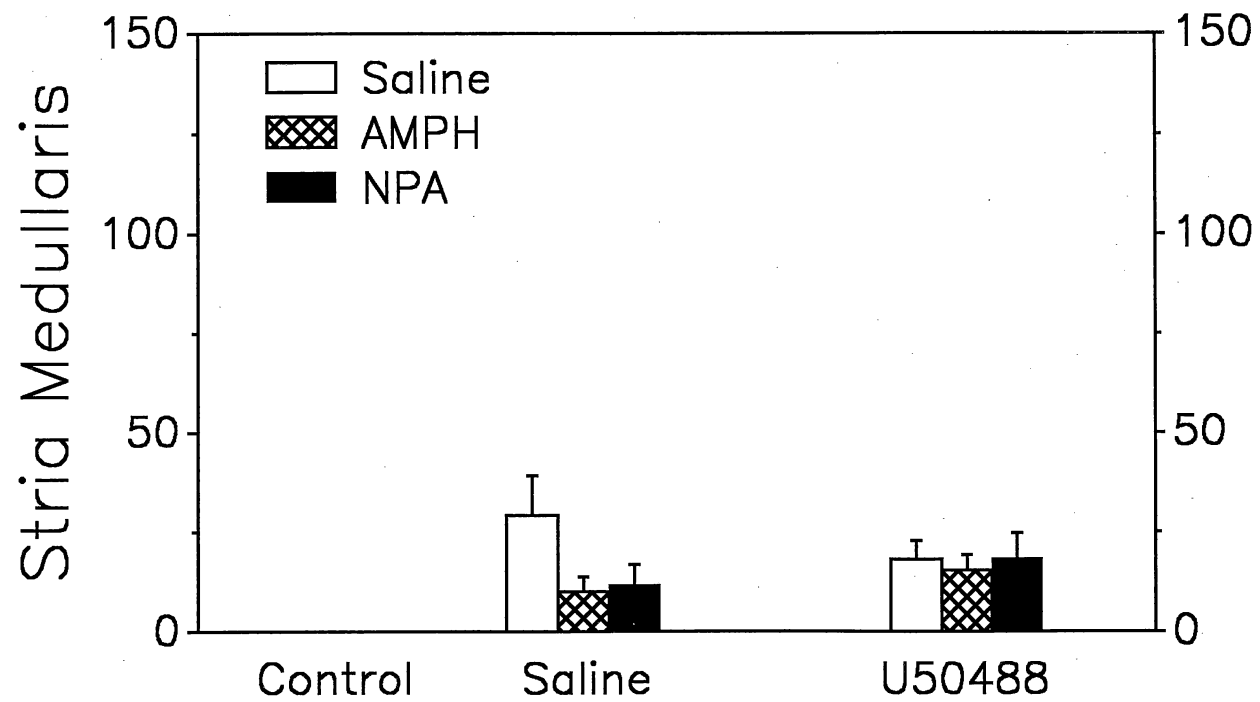


FIGURE CAPTION

Figure 32. Mean number (+SEM) of Fos-positive nuclei throughout the nucleus accumbens of the 80-day-old rat ($n = 6$). The rats were sacrificed 60 min after behavioral testing and were the same rats as those described in Figure 8.

80-Day-Olds

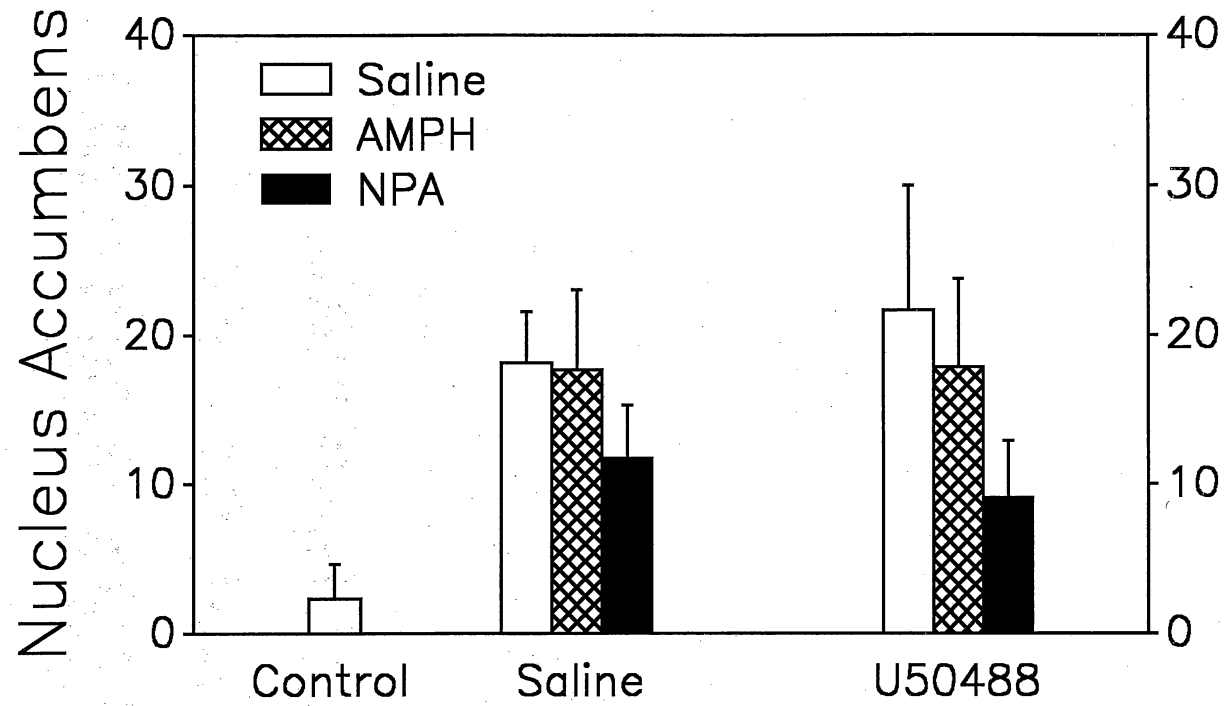


FIGURE CAPTION

Figure 33. Mean number (+SEM) of Fos-positive nuclei throughout the posterior hypothalamus of the 80-day-old rat ($n = 6$). The rats were sacrificed 60 min after behavioral testing and were the same rats as those described in Figure 8.

Posterior Hypothalamus

80-Day-Olds

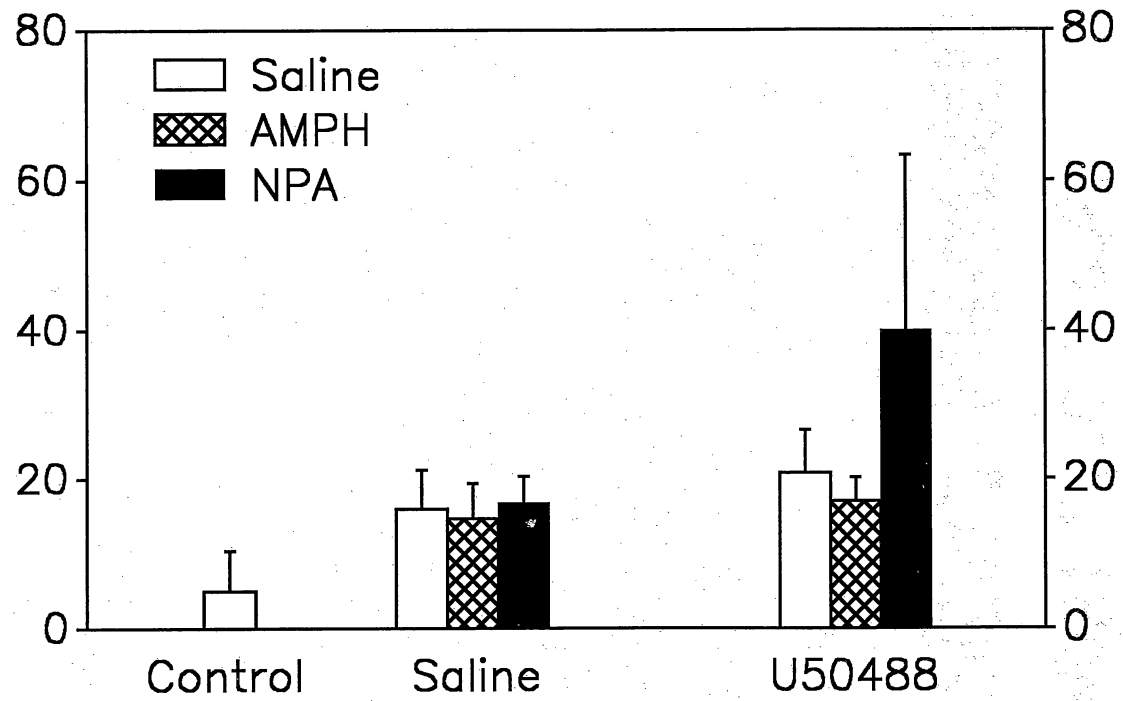
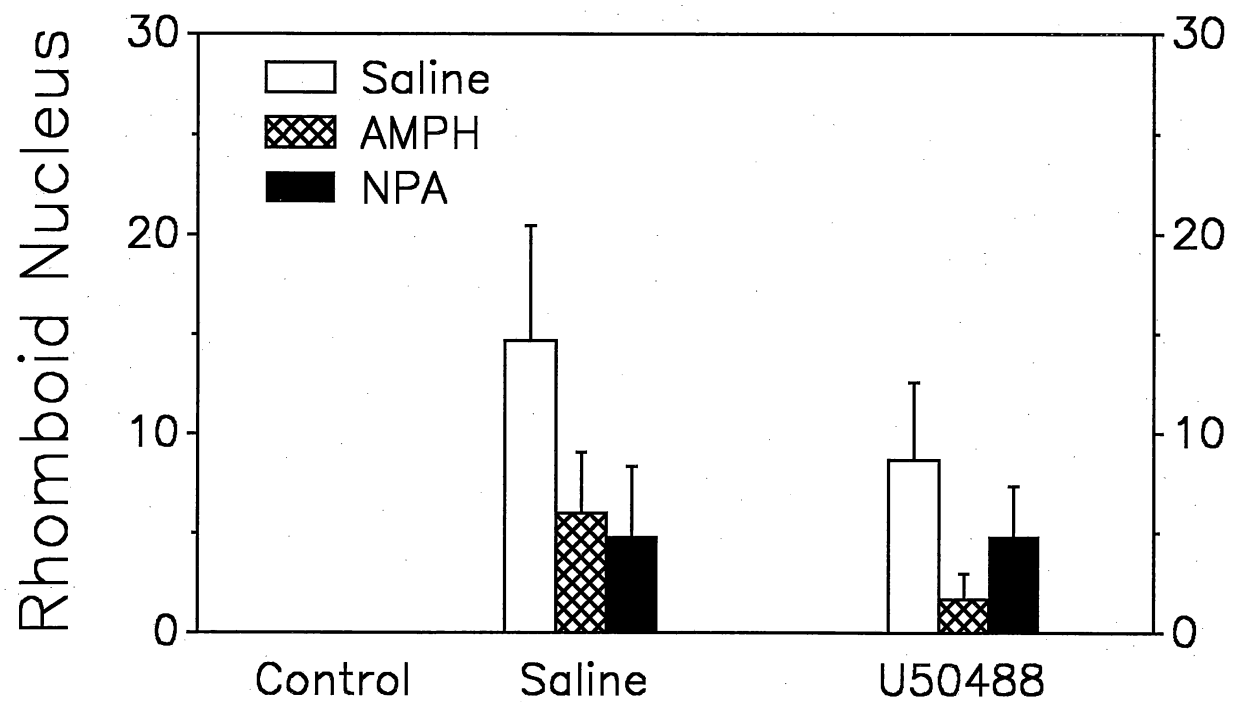


FIGURE CAPTION

Figure 34. Mean number (+SEM) of Fos-positive nuclei throughout the rhomboid nucleus of the 80-day-old rat ($n = 6$). The rats were sacrificed 60 min after behavioral testing and were the same rats as those described in Figure 8.

80-Day-Olds



Discussion

The 17-day-old rats treated with U-50,488 displayed an increased number of Fos-positive nuclei in several brain regions, suggesting locations where the DA and kappa opioid systems may interact. Interestingly, U-50,488 dramatically enhanced Fos expression in the striatum of the 17-day-old rat. This is particularly important because the striatum is known to be involved in the mediation of locomotor activity, rearing, and grooming (Arnt, 1987; Bordi et al., 1989). In the present study, NPA dramatically reduced U-50,488-induced Fos expression in the striatum. This pattern of neuronal activity correlates with behavior, as NPA depressed U-50,488-induced locomotor activity in the preweanling rat (see Experiment 3a). Similarly, amphetamine decreased U-50,488-induced Fos expression in the olfactory tubercles of the 17-day-old rat. The olfactory tubercles are involved in learning and memory and have been associated with conditioned place aversions (Ehret & Buckenmaier, 1994). Although speculative, it is possible that these Fos effects may be due to amphetamine attenuating U-50,488's aversive properties.

Several other brain regions of the 17-day-old rat displayed increased Fos immunoreactivity in response to U-50,488. Fos expression was enhanced by U-50,488 in

the cingulate cortex, septal area, and preoptic area. These brain regions mediate a variety of behaviors. For example, the septal area mediates analgesia, aversions, aggression, and locomotor activity (Nolte, 1981; Roeling et al., 1994; Schwarting & Huston, 1992). The cingulate cortex and preoptic area are involved in the mediation of emotion, memory, reproductive behaviors, and motoric functioning (Agmo & Villalpando, 1995; Koob & Swerdlow, 1988; Vogt, Finch, & Olson, 1992). However, common to each of these brain regions is participation in the modulation of locomotor activity. U-50,488's neuronal activation in these areas is consistent with the increased locomotor activity displayed after U-50,488 treatment in the 17-day-old rat (see Experiments 1a and 3a).

U-50,488 also enhanced Fos expression in the piriform cortex, habenula, amygdala, and septal area of the 17-day-old rat. These brain regions are involved in the mediation of a number of behaviors. For example, the piriform cortex and habenula mediate olfaction, conditioned taste aversions, analgesia, and sexual activity (Hasselmo, 1995; Lee & Huang, 1988; Werka, Skar, & Ursin, 1978). The amygdala and septal area are involved in conditioned place aversions, maternal behavior, analgesia, and aggression (Schwarting & Huston, 1992; Umino, Nishikawa, & Takahashi, 1995).

Overall, these brain areas share the common feature of being involved in the mediation of analgesia and aversions. It is known that kappa opioid agonists produce analgesia and aversions in the preweanling rat (Barr, 1992; Barr et al., 1986, 1994); therefore, it is not surprising that U-50,488 produced Fos expression in these brain regions.

Amphetamine and NPA also affected Fos immunoreactivity in the preweanling rat. Both of the DA agonists increased Fos expression in the piriform cortex, an area involved in the mediation of sniffing and exploratory behavior (Hasselmo, 1995; Nolte, 1981). Thus, amphetamine and NPA increase sniffing and locomotor activity in 17-day-old rats, while increasing Fos expression in the piriform cortex. Amphetamine also increased Fos induction in the cingulate cortex of the preweanling rat. Dopamine receptor activation in the cingulate cortex enhances locomotor activity (Agmo & Villalpando, 1995; Devinsky, Morrell, & Vogt, 1995).

With one exception, U-50,488 did not increase Fos immunoreactivity in 80-day-old rats. In the habenula, Fos expression was enhanced after U-50,488 treatment. Neuronal activation in the habenula is involved in olfaction, analgesia, sexual behaviors, and aversions. U-50,488 is known to produce both conditioned place and

taste aversions in the rat (Barr, 1992; Bechara & Van der Kooy, 1987; Lee & Huang, 1988; Thornton & Davies, 1991). Therefore, it is possible that U-50,488's aversive properties may be mediated in this area. Alternatively, U-50,488 attenuated NPA-induced sniffing, so it is possible that Fos expression in the habenula reflects sniffing or olfactory effects, rather than aversions.

Consistent with the antagonistic relationship displayed in the preweanling rat, U-50,488 also attenuated NPA-induced Fos expression in the piriform cortex of the adult rat. The piriform cortex is an olfactory relay center and this neuronal interaction between U-50,488 and NPA coincides with U-50,488's ability to depress NPA-induced sniffing (see Tables 3 and 6).

Treatment with DA agonists alone also affected Fos immunoreactivity in the 80-day-old rat. For example, amphetamine decreased Fos expression in the zona incerta of the adult rat, an area involved in the modulation of ingestive behaviors (Ricardo, 1981; Tonelli & Chairaviglio, 1995). NPA reduced Fos expression in the olfactory tubercles of the 80-day-old rat. The olfactory tubercles are involved in mediating sniffing and exploratory behavior (Hasselmo, 1995). The fact that amphetamine and NPA affected Fos expression in

these brain areas is not surprising as these regions are densely populated with dopamine receptors (Deutch, Tam, & Roth, 1985; Leibowitz & Rossokis, 1979; Stein, Carr, & Simon, 1990).

In summary, a number of brain regions showed drug-induced Fos expression. Although we attempted to make comparisons between regional Fos expression and behavior, it is important to realize that such comparisons must be interpreted with a great deal of caution. The brain regions showing Fos activity mediate a variety of behaviors and, in many cases, it is very speculative to link drug-induced behavioral effects with Fos. Even so, U-50,488 did dramatically enhance Fos expression in the striatum of the 17-day-old rat, an effect blocked by NPA treatment. This pattern of Fos activity is consistent with NPA's effects on U-50,488-induced locomotor activity (see Experiment 3a). When considered together, these results suggest that the striatum may be involved in the mediation of U-50,488's locomotor activating effects.

Experiment 4

The Behavioral Effects of NPA on U-50,488-Induced Behaviors in 17-Day-Old Rats

It was predicted that NPA and amphetamine would potentiate U-50,488-induced locomotor activity in the preweanling rat. In Experiment 3, however, a single dose of NPA was found to attenuate U-50,488-induced behaviors in the 17-day-old rat, while amphetamine left these behaviors unaffected. A possible explanation for these data is that combined treatment with U-50,488 and NPA produced intense stereotypical behaviors not measured in the last experiment. To assess this possibility, U-50,488-pretreated rats received lesser doses of NPA that should produce locomotor activity without inducing stereotypical behaviors.

Methods

Subjects. Eighty male and female 17-day-old Sprague-Dawley albino rats (Harlan) were used.

Procedures. Ten groups of 17-day-old rats ($n = 8$) received an acute injection of U-50,488 (5.0 mg/kg, s.c.) immediately prior to a 60-min behavioral testing session. Rats then received an acute injection of NPA (0.0, 0.001, 0.01, 0.1, or 1.0 mg/kg, i.p.) 30 min into the testing session.

Results

Line-crosses. During the initial three testing

blocks rats given U-50,488 had significantly more line-crosses than saline-treated rats (left panel, Figure 35) [pre main effect, $F(1,78)=470.08$, $P<0.001$]. During the final three testing blocks, NPA attenuated U-50,488-induced line-crosses in a dose-dependent manner (upper graph, right panel, Figure 35) [Pre X Post interaction, $F(4,70)=12.02$, $P<0.001$; and Tukey tests, $P<0.05$]. For instance, the 1.0 mg/kg dose of NPA produced the greatest decline in U-50,488-induced locomotor activity, with 0.1 and 0.01 mg/kg NPA producing a moderate decline in activity (see upper graph, right panel, Figure 35) [Tukey tests, $P<0.05$].

In contrast, when given alone, NPA enhanced the locomotor activity of the 17-day-old rats (see lower graph, right panel, Figure 35) [Pre X Post interaction, $F(4,70)=12.02$, $P<0.001$; and Tukey tests, $P<0.05$]. The 0.01 mg/kg NPA produced the most line-crosses, with 0.1 and 1.0 mg/kg NPA producing a moderate amount of activity (Tukey tests, $P<0.05$).

Stereotyped sniffing. During the first testing period, sniffing was not affected by U-50,488 pretreatment [U-50,488: 2.00 ± 0.36 ; saline: 1.07 ± 0.23]. Overall, U-50,488-pretreated rats had significantly fewer sniffing counts than saline-pretreated rats (see Table 8) [pre main effect, $F(1,79)=82.24$, $P<0.001$]. Saline-pretreated rats given

0.01, 0.1, or 1.0 mg/kg NPA had significantly more sniffing counts than rats receiving saline [Pre X Post interaction, $F(4,79)=13.82$, $P<0.001$; and Tukey tests, $P<0.05$]. U-50,488-pretreated rats given 0.01, 0.1, and 1.0 mg/kg NPA had significantly fewer sniffing counts than similarly treated saline-pretreated rats [Tukey tests, $P<0.05$].

FIGURE CAPTION

Figure 35. Mean number of line-crosses during the 60-min behavioral testing session. The 17-day-old rats ($n = 8$) were injected with U-50,488 (5.0 mg/kg, s.c.) or saline immediately prior to behavioral testing. Rats then received NPA (0.001, 0.01, 0.1, or 1.0 mg/kg, i.p.) or saline 30 min into the testing session.

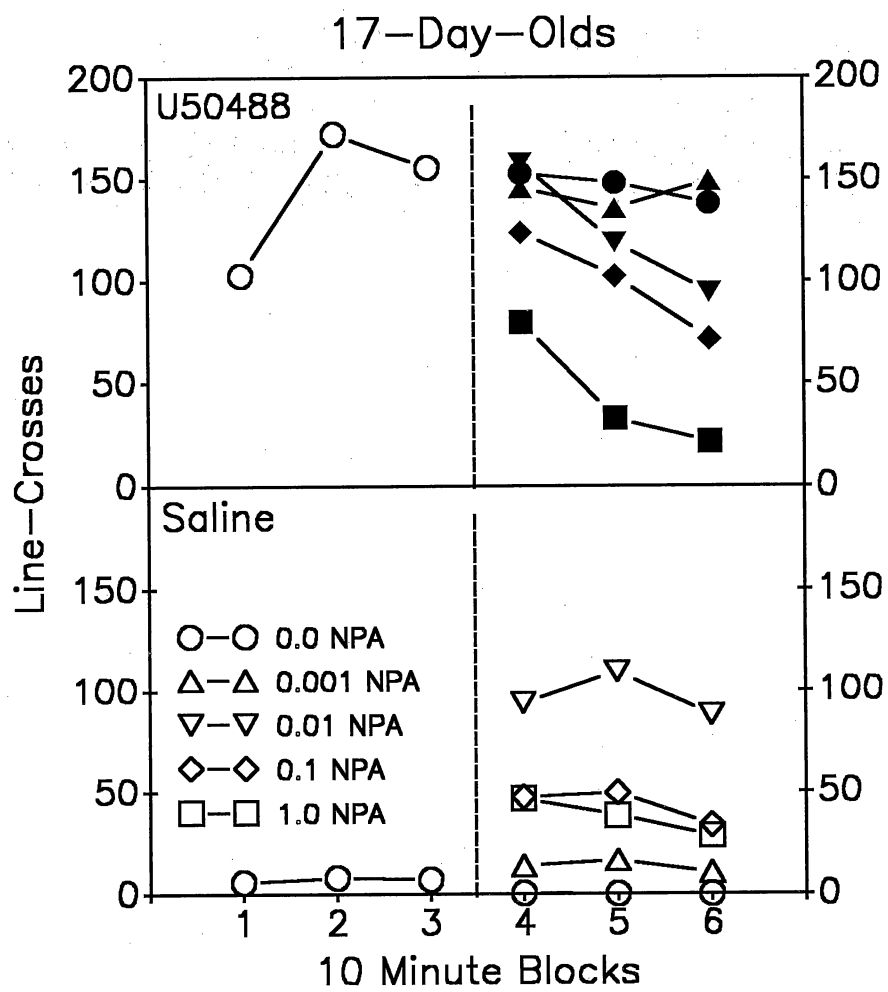


Table 8. Mean stereotyped sniffing counts (\pm SEM) of 17-day-old rats following treatment with NPA (0.0-1.0 mg/kg) 30 min after an initial injection of U-50,488 (5.0 mg/kg). Behavioral testing occurred immediately after U-50,488 treatment and was recorded for 60-min (divided into two 30-min testing periods). Data reported are from the second 30-min testing period. All doses are in mg/kg.

Drug Treatment	Stereotyped Sniffing Counts
Saline/Saline	0.00 \pm 0.00
U-50,488/Saline	0.37 \pm 0.37
Saline/0.001 NPA	5.37 \pm 1.59
U-50,488/0.001 NPA	2.87 \pm 1.34
Saline/0.01 NPA	30.50 \pm 6.36 ^{a b}
U-50,488/0.01 NPA	3.12 \pm 1.25
Saline/0.1 NPA	48.75 \pm 4.80 ^{a b}
U-50,488/0.1 NPA	8.62 \pm 5.52
Saline/1.0 NPA	61.37 \pm 5.67 ^{a b}
U-50,488/1.0 NPA	16.12 \pm 5.31

^aSignificant Pre X Post interaction indicating that saline-pretreated rats given NPA were significantly different from the saline/saline group, $P < 0.05$.

^bSignificant Pre X Post interaction indicating that saline-pretreated rats given NPA were significantly different from similarly treated U-50,488-pretreated rats, $P < 0.05$.

Discussion

NPA increased stereotyped sniffing in saline-pretreated rats in a dose-dependent manner: an effect attenuated by U-50,488-pretreatment. Conversely, NPA produced a dose-dependent *decrease* in U-50,488-induced locomotor activity. Thus, contrary to the original hypotheses, these results suggest an antagonistic relationship between U-50,488 and NPA.

Experiment 5

The Behavioral Effects of Amphetamine on U-50,488-Induced Behaviors in 17-Day-Old Rats

Methods

Subjects. Eighty male and female 17-day-old Sprague-Dawley albino rats (Harlan) were used.

Procedures. Ten groups of 17-day-old rats ($n = 8$) received an acute injection of U-50,488 (5.0 mg/kg, s.c.) immediately prior to a 60-min behavioral testing session. Rats then received an acute injection of amphetamine (0.0, 1.0, 2.5, 5.0, 10.0 mg/kg, i.p.) 30 min into the behavioral testing session.

Results

Line-crosses. Overall, there was a general decline in the total number of line-crosses across the final three testing blocks (see upper graph, Figure 36) [time main effect, $F(2,70)=12.13$, $P<0.001$; and Tukey tests, $P<0.05$]. However, the various doses of amphetamine had inconsistent effects on U-50,488-induced locomotor activity. For example, during the fourth testing block, 1.0, 2.5, and 10.0 mg/kg amphetamine produced significantly more line-crosses than saline; whereas, rats given 5.0 mg/kg amphetamine had fewer line-crosses than rats receiving 1.0 or 10.0 mg/kg amphetamine (see upper graph, right panel, Figure 36) [Post X Time interaction, $F(8,70)=6.01$, $P<0.001$; and Tukey tests,

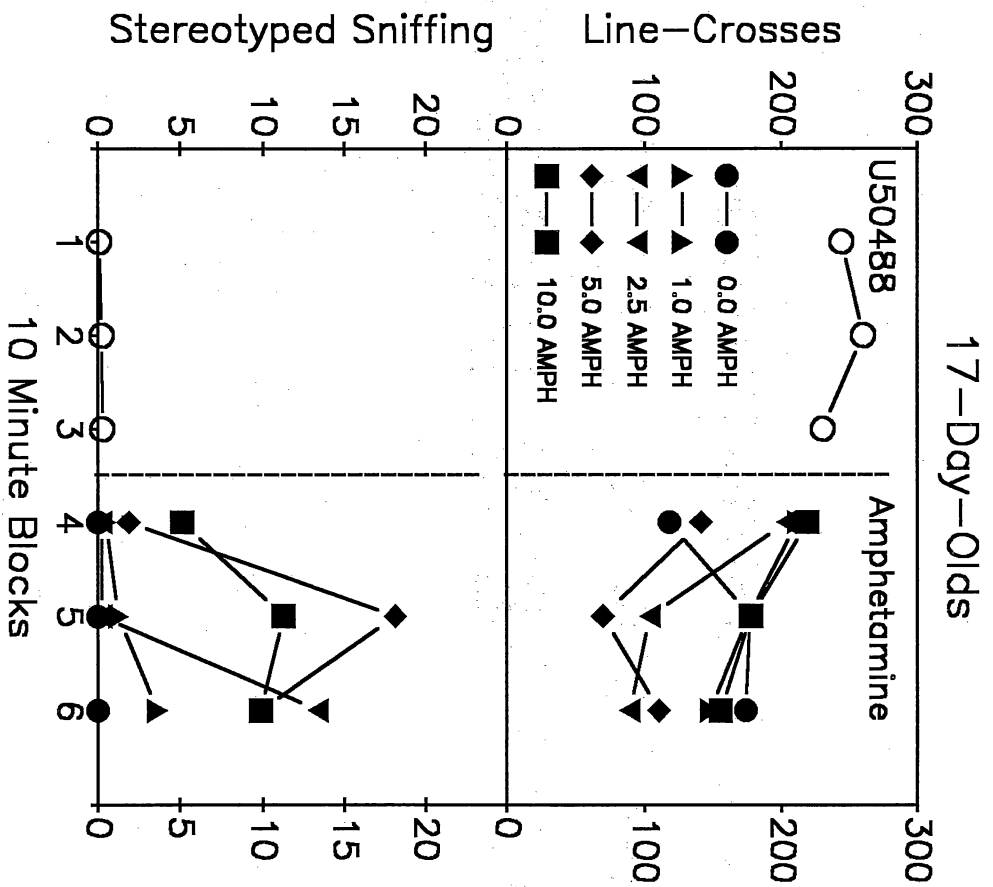
$P<0.05$). During the fifth testing block, U-50,488-pretreated rats given 2.5 or 5.0 mg/kg amphetamine had significantly fewer line-crosses than rats receiving saline, 1.0, or 10.0 mg/kg amphetamine [Tukey tests, $P<0.05$]. However, by the final testing block, only rats given 2.5 mg/kg amphetamine had significantly fewer line-crosses than rats receiving saline. During the final testing block there were no significant differences between the various groups of amphetamine-treated rats.

Stereotyped sniffing. Overall, amphetamine enhanced the stereotyped sniffing of the U-50,488-pretreated rats (see right panel, lower graph, Figure 38) [post main effect, $F(4,35)=15.30$, $P<0.001$; and Tukey tests, $P<0.05$]. There was a progressive increase in stereotyped sniffing during the final three testing blocks [time main effect, $F(2,70)=24.26$, $P<0.001$; and Tukey tests, $P<0.05$]. During the fourth testing block there were no significant differences between rats given amphetamine or saline. However, during the fifth and sixth testing block rats given 5.0 and 10.0 mg/kg amphetamine had significantly more sniffing counts than rats given saline (see right panel, lower graph, Figure 38) [Post X Time interaction, $F(8,70)=11.54$, $P<0.001$; and Tukey tests, $P<0.05$]. Not until the sixth testing block did 2.5 mg/kg amphetamine produce significantly

more sniffing than the saline group [Tukey tests,
 $P < 0.05$].

FIGURE CAPTION

Figure 36. Mean number of line-crosses and stereotyped sniffing counts during the 60-min behavioral testing session. The 17-day-old rats ($n = 8$) were injected with U-50,488 (5.0 mg/kg, s.c.) immediately prior to behavioral testing. Rats were then given amphetamine (1.0, 2.5, 5.0, or 10.0 mg/kg, i.p.) or saline 30 min into the testing session.



Discussion

Amphetamine produced no definitive pattern of action on U-50,488-induced line-crossing. For example, during the fourth and fifth testing blocks 1.0 mg/kg amphetamine produced more locomotor activity than 5.0 mg/kg amphetamine. By the final testing block there were no significant differences between amphetamine-treated rats. Therefore, while NPA appears to antagonize U-50,488-induced behaviors, amphetamine's actions on U-50,488-induced behaviors are more uncertain. To further examine how the DA and kappa systems interact two subsequent experiments were done. Rats were given amphetamine or NPA for five consecutive days. Following a two day wash-out period, chronically treated rats received a challenge injection of U-50,488 and were tested for cross-sensitization. If cross-sensitization were found between the DA agonists and U-50,488 this would indicate that the behavioral actions of these drugs are mediated through common dopaminergic mechanisms.

Experiment 6

Cross-Sensitization in the Preweanling Rat:

The Behavioral Effects of U-50,488

Following Chronic Treatment with NPA

The goal of this study was to determine if kappa opioid and DA agonists enhance locomotor activity through the same mechanisms. In Experiment 6, cross-sensitization was used to further assess this hypothesis. Sensitization, apparent in both preweanling and adult rats, is characterized by a progressive enhancement of the behavior-activating effects of DA or opiate agonists (see McDougall, Duke, Bolanos, & Crawford, 1994; Robinson & Becker, 1988; Vezina & Stewart, 1989). Several studies indicate that behavioral sensitization to DA and opiate agonists is produced by modifications in the functioning of nigrostriatal DA neurons (see Hoffman & Wise, 1992; Vezina & Stewart, 1989). For example, amphetamine-sensitized rats show enhanced striatal DA release in response to amphetamine challenge (Kalivas, 1985; Robinson & Becker, 1986). Similarly, rats sensitized to morphine show enhanced release, synthesis, and metabolism of DA in the nucleus accumbens following an acute injection of morphine (Kalivas, 1985; Kalivas & Duffy, 1987).

Interestingly, some drugs will cross-sensitize to

one another, suggesting that both drugs are acting on the same systems. For example, rats sensitized with GBR 12909 (a DA uptake inhibitor) show cross-sensitization in response to cocaine (Baldo & Kelly, 1991; Tolliver & Carney, 1994). Similarly, amphetamine-sensitized rats display a sensitized response to cocaine challenge (Kalivas & Weber, 1988). Importantly, opiate and DA agonists cross-sensitize to one another. For example, successive intra-ventral tegmental area injections of amphetamine result in a sensitized response to systemic morphine, an effect that is reciprocal (Stewart & Vezina, 1987; Vezina & Stewart, 1990). Since amphetamine appears to induce behavioral sensitization by modulating striatal DA functioning, morphine's ability to cross-sensitize suggests that these drugs are ultimately working through the same system. Therefore, if U-50,488 cross-sensitizes with the DA agonists, this would provide evidence that these drugs work through the same mechanisms.

Methods

Subjects. Thirty-two male and female 17-day-old Sprague-Dawley albino rats (Harlan) were used.

Procedures. Four groups of 11-day-old rats ($n = 8$) received injections of NPA (0.0 or 1.0 mg/kg, i.p.) for five consecutive days. Cross-sensitization was examined two days later as the preweanling rats (i.e., at 17 days

of age) received an acute injection of U-50,488 (5.0 mg/kg, s.c.) or NPA (1.0 mg/kg, i.p.). Circling was added to the behaviors being recorded. A circle count was recorded when the rat turned 360°, with the rear paws as the center point of the circle. Each day a 60-min behavioral testing session took place immediately after drug treatment.

Statistics. To analyze the behavioral data, repeated measure ANOVAs were performed across the five days of conditioning, with a separate ANOVA performed for the test day. In order to assess whether NPA produced behavioral sensitization a series of *t*-tests ($P < 0.05$) were performed to examine how the saline- and NPA-conditioned rats responded to a challenge dose of NPA.

Results

Conditioning: Effects of NPA on locomotor activity. Rats receiving NPA had significantly more line-crosses than saline controls across conditioning (see Figure 37) [conditioning main effect, $F(1,30)=62.48$, $P < 0.001$]. Furthermore, NPA produced a progressive increase in line-crosses from the first to last day of conditioning (upper graph, Figure 37) [Conditioning X Day interaction, $F(4,120)=29.19$, $P < 0.001$; and Tukey tests, $P < 0.05$].

Testing: Effects of U-50,488 challenge on locomotor

activity in NPA conditioned rats. On the test day sensitization was apparent, as rats given NPA during both conditioning and testing had significantly more line-crosses than rats given NPA on only the test day (see triangles, right panel, Figure 37) [$t(14)=2.58$, $P<0.05$]. U-50,488 did not cross-sensitize with NPA, since the two groups challenged with U-50,488 responded similarly on the test day (see boxes, right panel, Figure 37). On the test day, rats given U-50,488 had significantly more line-crosses than rats given NPA (see right panel, Figure 37) [Condition X Test interaction, $F(1,31)=7.28$, $P<0.05$; and Tukey tests, $P<0.05$].

Conditioning: Effects of NPA on stereotyped sniffing. Overall, rats receiving NPA had significantly more stereotyped sniffing counts than saline-treated rats (see Figure 38) [conditioning main effect, $F(1,30)=37.47$, $P<0.001$]. NPA produced a progressive increase in sniffing over the last three days of conditioning (upper graph, Figure 38) [Conditioning X Day interaction, $F(4,120)=38.17$, $P<0.001$; and Tukey tests, $P<0.05$].

Testing: Effects of U-50,488 challenge on stereotyped sniffing in NPA-conditioned rats. On the test day, NPA-conditioned rats displayed a sensitized response to NPA. Specifically, when challenged with NPA, rats conditioned with NPA had significantly more

stereotyped sniffing counts than rats conditioned with saline (see triangles, right panel, Figure 38) [$t(14)=3.01$, $P<0.01$]. On the test day, regardless of conditioning, U-50,488 challenge failed to produce any stereotyped sniffing. Thus, rats given NPA on the test day had significantly more stereotyped sniffing counts than rats receiving U-50,488 (see right panel, Figure 38) [Conditioning X Test interaction, $F(1,31)=9.45$, $P<0.05$; and Tukey tests, $P<0.05$].

Conditioning: Effects of NPA on circling. During conditioning, NPA produced a small, but significant, increase in circling when compared to saline-treated rats (upper graph, Figure 39) [conditioning main effect, $F(1,16)=11.64$, $P<0.05$].

Testing: Effects of U-50,488 challenge on circling in NPA-conditioned rats. On the test day, rats conditioned with NPA failed to display a sensitized circling response after NPA challenge (see triangles, upper right panel, Figure 39) [$t(14)=1.08$, $P>0.05$]. When given alone, U-50,488 failed to produce circling; however, NPA-conditioned rats challenged with U-50,488 had significantly more circling counts than all other groups (see box, upper right panel, Figure 39) [Conditioning X Test interaction, $F(1,24)=21.42$, $P<0.001$; and Tukey tests, $P<0.05$].

FIGURE CAPTION

Figure 37. Mean number of line-crosses of rats given five daily injections of NPA (1.0 mg/kg, i.p.) or saline starting at 11 days of age ($n = 8$). At 17 days of age (i.e., after a 2-day interval), rats given successive saline or NPA injections were then given a single challenge injection of U-50,488 (5.0 mg/kg, s.c.) or NPA (1.0 mg/kg, i.p.).

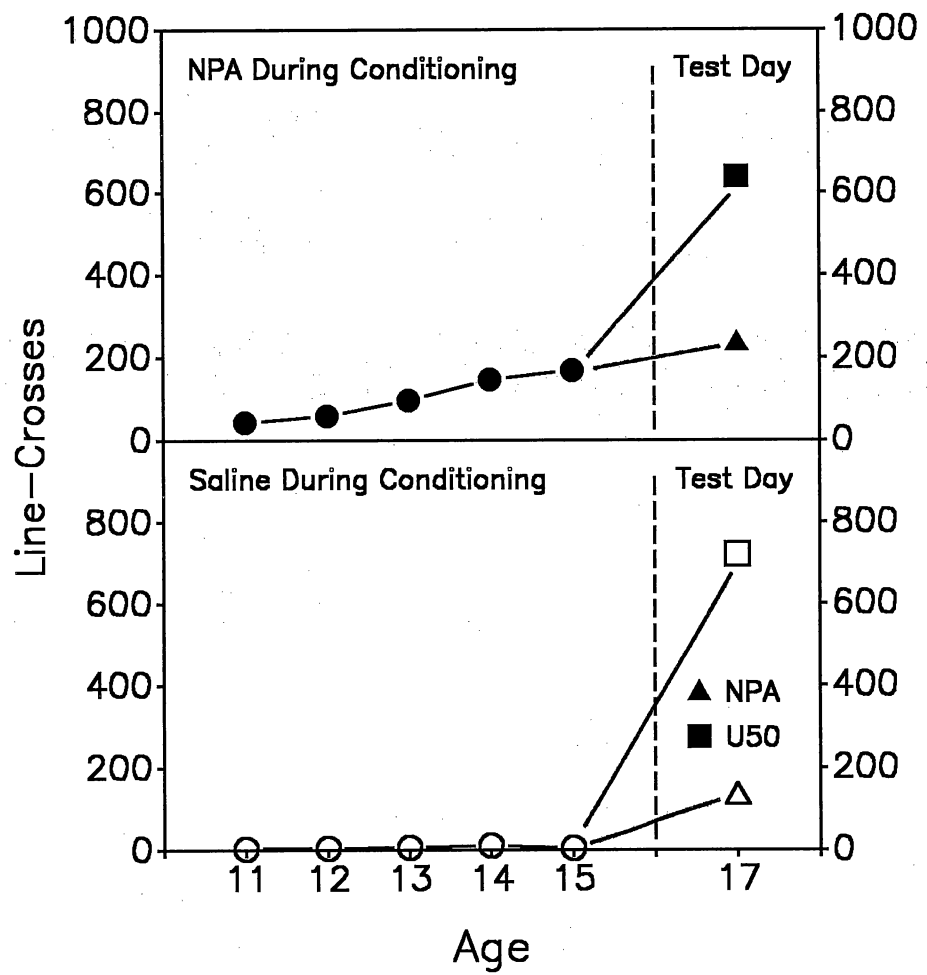


FIGURE CAPTION

Figure 38. Mean number of stereotyped sniffing counts of rats given five daily injections of NPA (1.0 mg/kg, i.p.) or saline starting at 11 days of age ($n = 8$). At 17 days of age (i.e., after a 2-day interval), rats given successive saline or NPA injections were then given a single challenge injection of U-50,488 (5.0 mg/kg, s.c.) or NPA (1.0 mg/kg, i.p.).

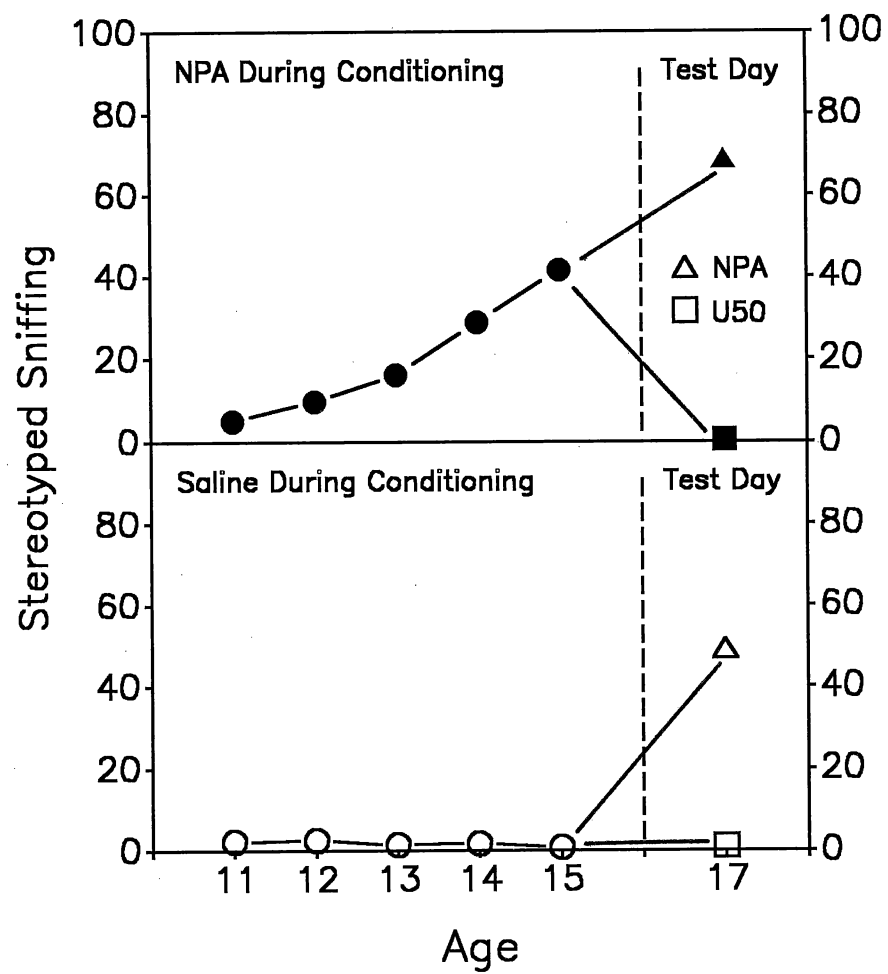
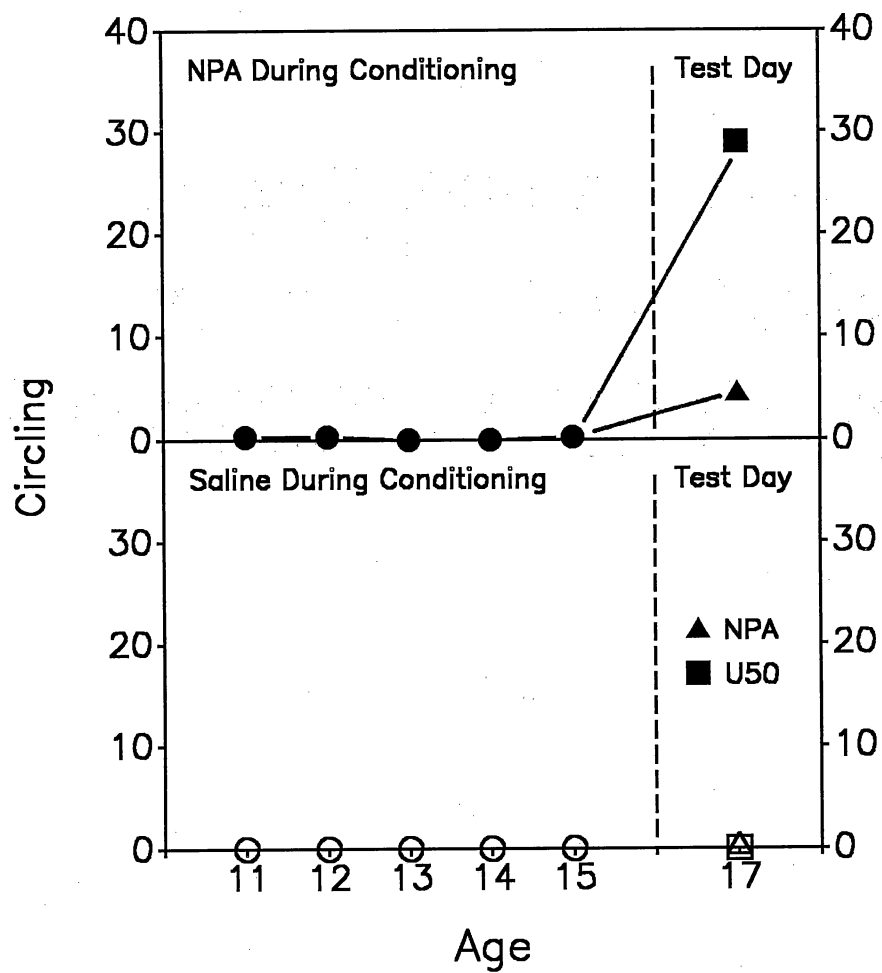


FIGURE CAPTION

Figure 39. Mean number of circling counts of rats given five daily injections of NPA (1.0 mg/kg, i.p.) or saline starting at 11 days of age ($n = 8$). At 17 days of age (i.e., after a 2-day interval), rats given successive saline or NPA injections were then given a single challenge injection of U-50,488 (5.0 mg/kg, s.c.) or NPA (1.0 mg/kg, i.p.).



Discussion

No evidence for cross-sensitization between NPA and U-50,488 was apparent. On the test day, rats conditioned with NPA did display sensitized locomotor activity and stereotyped sniffing. Interestingly, U-50,488 challenge significantly enhanced circling in NPA-conditioned rats. These results support the findings of earlier experiments (i.e., Experiments 3a and 4) suggesting that a generally antagonistic relationship exists between U-50,488 and NPA.

Experiment 7

Cross-Sensitization in the Preweanling Rat:

The Behavioral Effects of U-50,488

Following Chronic Treatment with Amphetamine

Methods

Subjects. Thirty-two male and female 17-day-old Sprague-Dawley albino rats (Harlan) were used.

Procedures. Four groups of 11-day-old rats ($n = 8$) received injections of amphetamine (0.0 or 2.5 mg/kg, i.p.) for five consecutive days. Cross-sensitization was examined two days later as the preweanling rats (17 days old) received an acute injection of U-50,488 (5.0 mg/kg, s.c.) or amphetamine (2.5 mg/kg, i.p.). Each day a 60-min behavioral testing session began immediately drug treatment.

Statistics. To analyze the behavioral data, repeated measure ANOVAs were performed across the five days of conditioning, with a separate ANOVA performed for the test day. In order to assess whether amphetamine produced behavioral sensitization a series of t -tests ($P < 0.05$) were performed to examine how the saline- and amphetamine-conditioned rats responded to a challenge dose of amphetamine.

Results

Conditioning: Effects of amphetamine on locomotor activity. Overall, rats receiving repeated treatments

with amphetamine had significantly more line-crosses than saline controls on each day of conditioning (see Figure 40) [Conditioning X Day interaction, $F(4,120)=95.34$, $P<0.001$; and Tukey tests, $P<0.05$].

Amphetamine produced a progressive increase in locomotor activity across days (upper graph, Figure 40) [Tukey tests, $P<0.05$].

Testing: Effects of U-50,488 challenge on locomotor activity in amphetamine-conditioned rats. On the test day, amphetamine-conditioned rats failed to display sensitized locomotor activity following amphetamine challenge (see triangles, right panel, Figure 40) [$t(14)=1.57$, $P>0.05$]. Overall, rats challenged with U-50,488 had significantly more line-crosses than rats challenged with amphetamine [test main effect, $F(1,31)=34.66$, $P<0.001$]. On the test day, there were no significant differences in line-crosses between amphetamine- or saline-conditioned rats in response to U-50,488 challenge.

Conditioning: Effects of amphetamine on stereotyped sniffing. Rats treated with amphetamine had significantly more sniffing counts than saline controls on each day of conditioning (see Figure 41) [Conditioning X Day interaction, $F(4,120)=3.89$, $P<0.05$; and Tukey tests, $P<0.05$].

Testing: Effects of U-50,488 challenge on

stereotyped sniffing in amphetamine-conditioned rats.

On the test day, following amphetamine challenge, saline-conditioned rats had significantly more sniffing counts than amphetamine-conditioned rats (see triangles, right panel, Figure 41) [$t(14)=2.15$, $P<0.05$]. Overall, rats challenged with amphetamine on the test day had significantly more stereotyped sniffing counts than rats challenged with U-50,488 (see right panel, Figure 41) [Condition X Test interaction, $F(1,31)=4.26$, $P<0.05$].

Conditioning: Effects of amphetamine on circling.

Amphetamine did not produce any circling during conditioning (see Figure 42).

Testing: Effects of U-50,488 challenge on circling in amphetamine-conditioned rats. On the test day, amphetamine challenge failed to produce any circling. However, amphetamine-conditioned rats challenged with U-50,488 had significantly more circling counts than all other groups (see right panel, Figure 42) [Conditioning X Test interaction, $F(1,31)=20.59$, $P<0.001$; and Tukey tests, $P<0.05$].

FIGURE CAPTION

Figure 40. Mean number of line-crosses of rats given five daily injections of amphetamine (2.5 mg/kg, i.p.) or saline starting at 11 days of age ($n = 8$). At 17 days of age (i.e., after a 2-day interval), rats given successive saline or amphetamine injections were then given a single challenge injection of U-50,488 (5.0 mg/kg, s.c.) or amphetamine (2.5 mg/kg, i.p.).

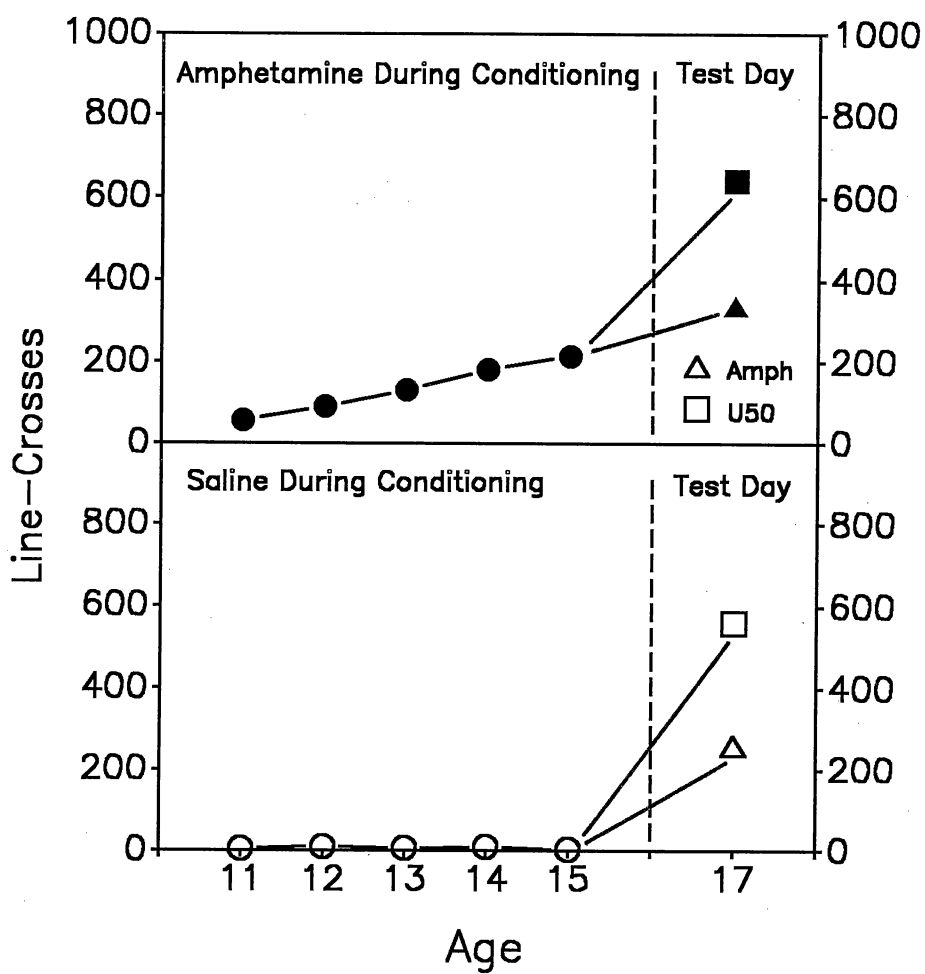


FIGURE CAPTION

Figure 41. Mean number of stereotyped sniffing counts of rats given five daily injections of amphetamine (2.5 mg/kg, i.p.) or saline starting at 11 days of age ($n = 8$). At 17 days of age (i.e., after a 2-day interval), rats given successive saline or amphetamine injections were then given a single challenge injection of U-50,488 (5.0 mg/kg, s.c.) or amphetamine (2.5 mg/kg, i.p.).

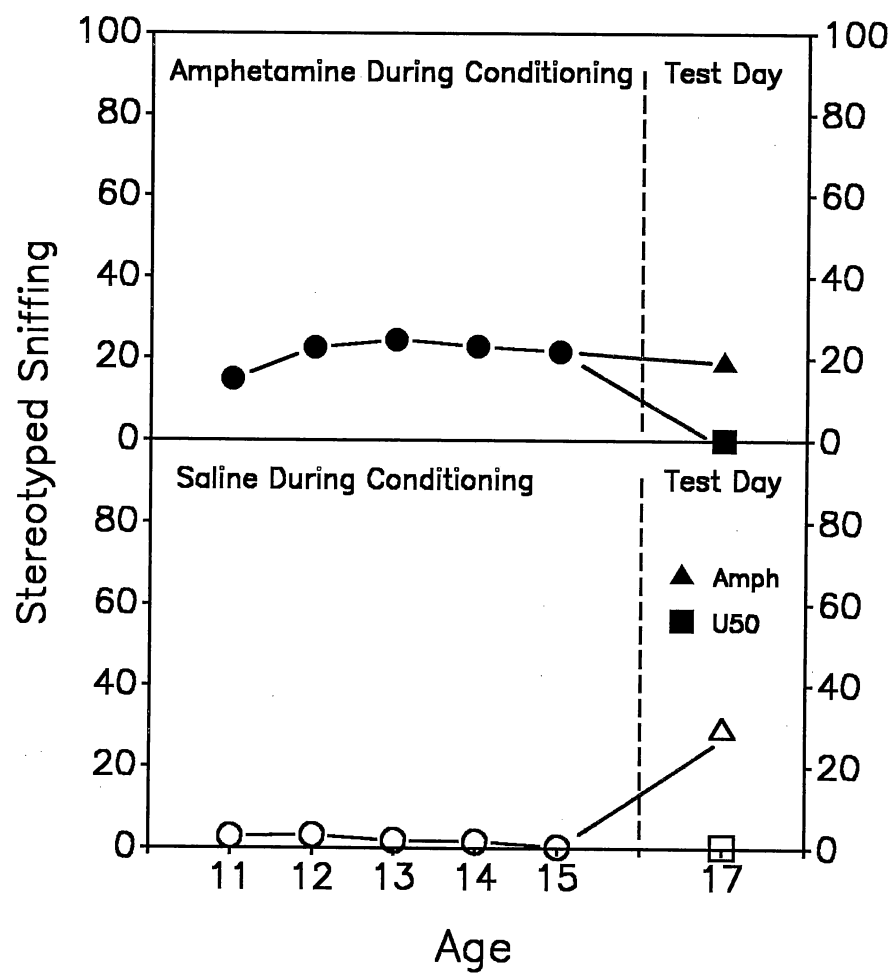
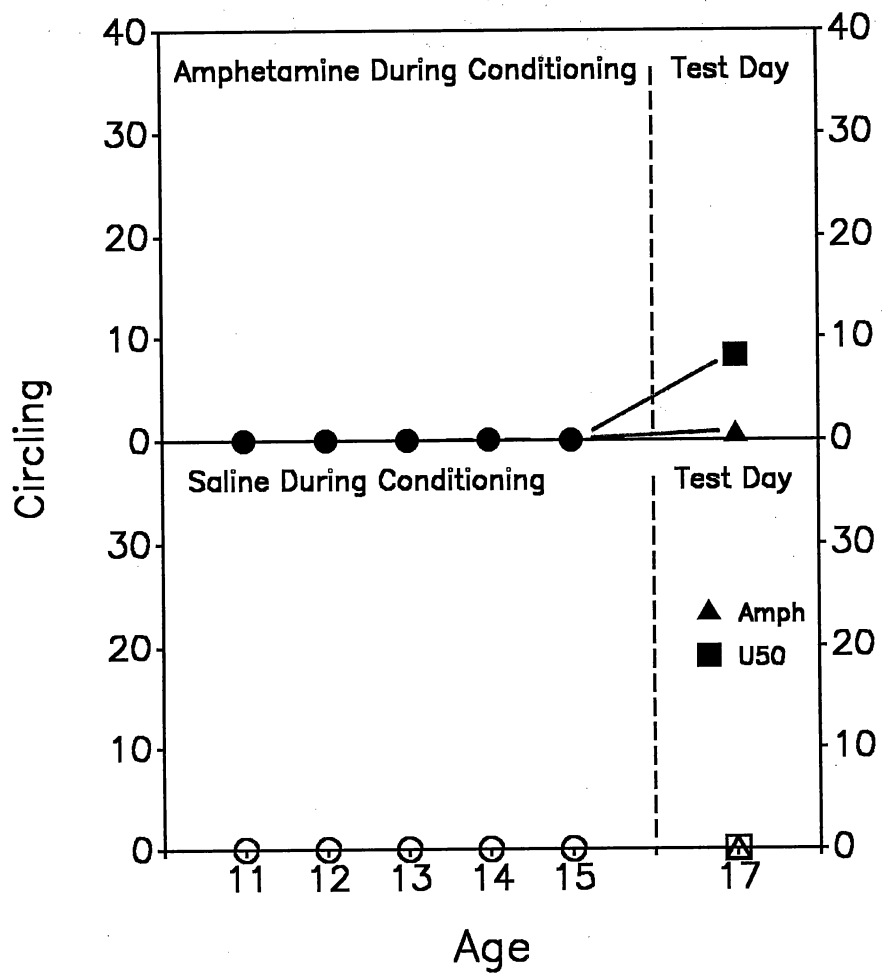


FIGURE CAPTION

Figure 42. Mean number of circling counts of rats given five daily injections of amphetamine (2.5 mg/kg, i.p.) or saline starting at 11 days of age ($n = 8$). At 17 days of age (i.e., after a 2-day interval), rats given successive saline or amphetamine injections were then given a single challenge injection of U-50,488 (5.0 mg/kg, s.c.) or amphetamine (2.5 mg/kg, i.p.).



Discussion

There was no evidence of cross-sensitization between U-50,488 and amphetamine. Rats challenged with U-50,488 showed a similar increase in locomotor activity regardless of whether they were conditioned with amphetamine or saline. Overall, these results suggest that U-50,488 and amphetamine increase locomotor activity by affecting different systems.

General Discussion

The purpose of the present study was to examine U-50,488's paradoxical effects on locomotor activity. More specifically, in the adult rat U-50,488 decreases locomotor activity; whereas, U-50,488 dramatically increases the locomotor activity of the preweanling rat. In these younger animals, the increased locomotor activity produced by U-50,488 is similar to that observed following DA agonist treatment. Therefore, the purpose of these experiments was to determine whether U-50,488's locomotor activating effects are mediated through a dopaminergic mechanism. Six hypotheses were originally proposed: (1) It was suggested that U-50,488 would decrease the locomotor activity of the 80-day-old rat. The results of Experiment 1d supported this hypothesis, as all doses (0.2--10.0 mg/kg) of U-50,488 reduced the locomotor activity of adult rats. (2) As predicted, U-50,488 increased the locomotor activity of the preweanling rat (see Figures 2 and 7). (3) It had been originally hypothesized that U-50,488 would potentiate amphetamine- and NPA-induced behaviors in the preweanling rat. However this was not substantiated, as NPA actually attenuated U-50,488-induced locomotor activity (see Figure 7). Amphetamine's actions were less clear, but the results suggest that amphetamine also depresses the behaviors

of U-50,488 treated rats. (4) Flupenthixol (a nonselective DA receptor antagonist) was predicted to reduce U-50,488-induced locomotor activity. The results of Experiment 2 supported this hypothesis, as flupenthixol produced a dose-dependent decline in U-50,488-induced activity (see Figure 6). (5) As predicted, amphetamine and NPA stimulated regional Fos expression in the preweanling rat. The areas showing the greatest amphetamine- and NPA-induced Fos immunoreactivity were the piriform cortex and cingulate cortex (see Figures 11 and 16). (6) In general, U-50,488-, amphetamine-, and NPA-induced Fos immunoreactivity did occur in the same brain regions. However, instead of U-50,488 potentiating NPA- and amphetamine-induced Fos, the DA agonists attenuated U-50,488-induced Fos in the striatum and olfactory tubercles (see Figures 9 and 10).

The results of the present study showed that the kappa opioid agonist U-50,488, the direct DA agonist NPA, and the indirect DA agonist amphetamine, all independently enhanced behavioral responding in the preweanling rat. High doses of the DA antagonist flupenthixol (0.4 and 0.8 mg/kg) reduced U-50,488-induced locomotor activity (see Figure 6). This suggests that U-50,488 may have been working via a dopaminergic mechanism. Subsequent experiments indicate

however, that flupenthixol's ability to block U-50,488-induced locomotor activity may have been due to a general depression of motoric functioning and not due to direct interactions with kappa neurons.

To better assess the kappa/DA interaction, U-50,488-pretreated 17-day-old rats were given amphetamine or NPA prior to behavioral testing. Although it was predicted that U-50,488 pretreatment would potentiate DA agonist-induced activity, acute treatment with amphetamine had little or no effect on U-50,488-induced behaviors. Surprisingly, acute treatment with NPA attenuated U-50,488-induced behavioral responding in the 17-day-old rat. More specifically, U-50,488 dramatically enhanced locomotor activity, while NPA reduced this activity in a dose-dependent manner. These findings were of particular interest because they indicate that kappa opioid neurons and DA neurons interact to modulate unlearned activity in the preweanling rat. However, instead of the hypothesized facilitatory mechanism, it appears that the kappa opioid and DA systems have an antagonistic relationship.

Consistent with this, there was little evidence of cross-sensitization between the kappa opioid agonist and either of the DA agonists. Specifically, U-50,488 challenge failed to elicit a cross-sensitized response in amphetamine- or NPA-conditioned rats. One exception

was that rats receiving NPA or amphetamine during conditioning displayed pronounced circling following U-50,488 treatment. This was not true cross-sensitization however, since the circling behavior did not show a sensitized response after repeated amphetamine or NPA treatment. Importantly, regardless of conditioning (i.e., NPA or amphetamine), U-50,488 produced a dramatic enhancement of locomotor activity on the test day that was similar to that of U-50,488 alone. Chronic pretreatment with NPA or amphetamine did not affect the enhanced locomotor activity that was produced by a challenge injection of U-50,488 (see right panel, Figures 37 and 40). These results are interesting, because acute treatment with NPA depressed U-50,488-induced locomotor activity in preweanling rats (see Figure 35). Overall, these results indicate that the locomotor activating actions of U-50,488 may not be mediated directly through a dopaminergic mechanism.

In addition to behavioral testing, the neuronal response to amphetamine and NPA following U-50,488 pretreatment was examined by measuring the induction of Fos, the protein product of the early response gene *c-fos*. Presumably, increased Fos immunoreactivity is the result of enhanced neuronal activity and, therefore, can be used to identify those brain regions involved in the mediation of behavior (Crawford et al., 1995; Graybiel

et al., 1990; Sharp, Sagar, & Swanson, 1993). In the 80-day-old rat, NPA reduced Fos expression in the olfactory tubercles. Conversely, NPA increased Fos induction in the piriform cortex, an effect attenuated by U-50,488 pretreatment. In general, NPA's effects on Fos expression are consistent with studies using the direct DA agonist apomorphine, which increased Fos immunoreactivity in the piriform cortex of the adult rat (Wirtshafter & Asin, 1995; Wirtshafter, Asin, & Pitzer, 1994). In the present study amphetamine reduced the amount of Fos expression in the zona incerta, while having only trivial effects in all other brain regions. The dose of amphetamine (2.5 mg/kg) used in Experiment 3c may be responsible for the lack of amphetamine-induced Fos expression. A higher dose of amphetamine (5.0 mg/kg) was shown to induce Fos expression in the striatum and substantia nigra of the adult rat (Jaber et al., 1995). With the exception of the habenula, U-50,488 alone did not enhance Fos expression in any of the brain regions examined. In general, this is consistent with the existing adult literature (Crawford et al., 1995).

In the preweanling rat, U-50,488, as well as amphetamine and NPA, produced Fos immunoreactivity. U-50,488 enhanced Fos expression in the striatum, olfactory tubercles, piriform cortex, habenula, preoptic

area, amygdala, stria medullaris, cingulate cortex, and septal area of the 17-day-old rat. Of special interest was the striatum, as U-50,488-induced locomotor activity was accompanied by dramatically increased Fos expression in the striatum. NPA treatment blocked both U-50,488-induced locomotion and striatal Fos expression. Thus suggesting that the striatum may be one of the brain regions mediating U-50,488's locomotor activating effects. The DA agonists also affected Fos immunoreactivity in other brain regions of the preweanling rat. For example, amphetamine depressed U-50,488-induced Fos expression in the olfactory tubercles; whereas, amphetamine and NPA enhanced Fos expression in the piriform cortex. These results indicate that while DA agonists can independently affect Fos immunoreactivity, these same DA agonists will modulate U-50,488-induced Fos expression in a number of brain regions.

Evidence suggests that kappa opioids have dual opposing effects on activity: a motor activating effect mediated by non-dopaminergic pars reticulata cells and a motor inhibitory effect mediated through the pars compacta DA cells (Matsumoto et al., 1988; Thompson & Walker, 1990). Receptor binding studies have indicated that large amounts of kappa opiate receptors are present in the striatum and pars compacta of the substantia

nigra, with low levels present in the pars reticulata (Matsumoto et al., 1988; Merchenthaler et al., 1986). Similarly, binding studies have found high levels of DA receptors in the striatum and pars compacta of the substantia nigra, with low level of DA receptors in the pars reticulata (Kreiss et al., 1995; Porter, Greene, Higgins, & Greenamyre, 1994; Spampinato, Gozlan, Daval, Fattaccini, & Hamon, 1988).

Therefore, based on these binding studies it is possible that the striatum is the brain region responsible for the present behavioral data. More specifically, it is possible that activation of the kappa opioid receptors in the pars compacta inhibits DA neurons projecting from the substantia nigra to the striatum. If true, this would account for U-50,488's ability to depress NPA-induced sniffing. Unfortunately, this does not account for NPA's ability to depress U-50,488-induced activity. At present there is no adequate explanation for this effect. In general, however, activation of kappa opioid receptors in the adult rat is associated with a reduction of DA-mediated behavior (Crawford et al., 1995; Di Chiara & Imperato, 1988; Matsumoto et al., 1988). Therefore, a similar antagonistic relationship between the kappa opioid and DA systems in the preweanling rat is not surprising.

In the cross-sensitization experiments, chronic

treatment with amphetamine or NPA did not affect U-50,488-induced locomotor activity (see Figures 37 and 40). However, U-50,488 challenge did produce circling in amphetamine- and NPA-conditioned preweanling rats (see Figures 39 and 42). Importantly, circling only occurred in rats that had received both U-50,488 and one of the DA agonists. Apparently circling is a kappa opioid mediated effect, but it is only expressed after prior treatment with NPA or amphetamine. Thus it is possible that normal dopaminergic functioning may mask circling, but that the neurobiological changes produced by chronic DA agonist treatment (i.e., changes in receptor numbers, G-proteins, receptor sensitivity) allowed U-50,488-induced circling to be expressed. Curiously, bilateral microinjections of amphetamine or apomorphine into the substantia nigra causes circling in rats (Jerussi & Glick, 1976). So it is possible that U-50,488 and the DA agonists combine to produce circling behavior by activating this brain region. Consistent with this, the substantia nigra is known to have an abundance of both kappa opioid and DA D₁ and D₂ receptors (Kreiss et al., 1995; Matsumoto et al., 1986).

It had been originally hypothesized that the interaction between the kappa opioid and DA systems in the preweanling rat was of a facilitatory nature: That is, it was predicted that U-50,488 would potentiate DA

agonist-induced locomotor activity. Instead, these systems seem to interact in an antagonistic fashion in the preweanling rat. Thus, this still leaves the basic question of why U-50,488 paradoxically increases the locomotor activity of the preweanling rat, while leaving the activity of the adult rat unaffected. Although the DA system appears to be eliminated as a possible explanation, it is conceivable that ontogenetic changes in NMDA receptors may be responsible for U-50,488's actions.

Dizocilpine, an NMDA receptor antagonist, produces a robust increase in locomotor activity in the preweanling rat (Duke, Bolanos, Garmsen, Clair, & McDougall, 1995; Scalzo & Burge, 1994). Evidence for a kappa/NMDA interaction includes: receptor binding studies showing that high levels of NMDA receptors are located in the striatum and substantia nigra of the rat (Carroll, Holloway, Brotchie, & Mitchell, 1995; Snell & Johnson, 1985). Second, dizocilpine, like U-50,488, increases Fos expression in the striatum (Liu, Nickolenko, & Sharp, 1994). And finally, dizocilpine produces no cross-sensitization in rats conditioned with amphetamine or NPA. Thus, dizocilpine's locomotor activating effects, like U-50,488's, do not appear to be mediated through a dopaminergic mechanism. Once again, U-50,488 and dizocilpine share the common feature that

they both dramatically increase the locomotor activity of preweanling rats well above that seen in adults. Therefore, although speculative, it is possible that U-50,488 and dizocilpine work through common mechanisms independent of actions on DA neurons.

In summary, the kappa opioid agonist, U-50,488, dramatically increases the locomotor activity of the preweanling rat. The striatum may be involved in the mediation of this activity, as enhanced striatal Fos expression accompanied the U-50,488-induced increase in locomotor activity. Importantly, the direct DA agonist NPA attenuated Fos expression in the striatum, as well as producing a dose-dependent decrease in U-50,488's locomotor activating effects. The opposite was also the case, NPA-induced sniffing was attenuated by U-50,488. When considered together, these results indicate that the DA and kappa systems have a generally antagonistic relationship with each other. The lack of cross-sensitization between U-50,488 and amphetamine or NPA provides further evidence indicating that U-50,488's locomotor activating effects are not mediated directly through a dopaminergic mechanism. One possibility is that U-50,488's locomotor activating effects may be mediated through a non-dopaminergic mechanism in the striatum.

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